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Ion-selective electrodes : applications in the clinical chemistry laboratory.

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ION-SELECTIVE ELECTRODES:

APPLICATIONS IN THE CLINICAL CHEMISTRY LABORATORY

by

GODFREY CORNELIUS MOSES

A Major Clinical Chemistry Critique
Submitted to the Faculty of Graduate Studies
through the Department of Chemistry in
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1977

ABSTRACT

The basic principles, modes of operation, types and fabrication of ion-selective electrodes in general are reviewed. The application of some of these sensors in the Clinical Chemistry Laboratory and clinical significance of their measurements are also discussed.

Some experimental data for the Orion ammonia and ionized calcium electrodes are reported.

Comparison studies between the automated analysis of plasma ammonia and both the Ion-exchange resin and the enzymatic methods have been performed. Good correlation has been obtained for the ion-exchange resin method, whereas the correlation for the enzymatic method is not satisfactory.

Similar studies between ionized calcium measured by the Orion Calcium Ionalyzer® and the normogram method for ionized calcium indicated a poor correlation between the two methods. Precision and percent recoveries for the automated analysis of ammonia by Ion-selective electrode are well within acceptable limits.

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I also wish to thank my many friends in the Chemistry Department at Grace Hospital for their co-operation through-out this course.

I also wish to extend my gratitude to Dr. N. F. Taylor for his advice given from time to time.

Finally, I would like to thank my wife Sheila for the many ways in which she has helped through-out this study.

DEDICATION

TO

MY WIFE

SHEILA.

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ABREVIATIONS

ISE	ION-SELECTIVE ELECTRODE(S).
In	Natural logarithm.
mV	Millivolt.
Redox	Reduction-oxidation process.
PVC	Polyvinyl chloride.
PE	Polyethylene.
BUN	Blood urea nitrogen.
mM	Millimolar.
M	Molar.
LDH	Lactate dehydrogenase.
L-AAO	L-Amino acid oxidase.
D-AAO	D-Amino acid oxidase.
mosmol	Milliosmoality.
Kg	Kilogram.
µg/dl	Microgram per deciliter
mg/dl	Milligram per deciliter
M/L	Moles per liter.
ml	Milliliter.
meq/L	Milliequivalent per liter.
µl	Microliter.
µg	Microgram.
L	Liter.
SD	Standard deviation.
%CV	Percent coefficient of variation.

INTRODUCTION

Ion-selective electrodes are membrane electrodes which permit the measurement of ionic activities in solutions with a high degree of selectivity. The term ion-specific is less preferred to the term ion-selective because these electrodes are rarely specific in their response to one ionic species over others, although this is a desirable property sought when new electrodes are being designed. A membrane in this context is used to denote a thin section of electrically conducting material separating two solutions and across which a potential develops. It should not be confused with biological membranes or with the permeability aspect of a membrane through which a solute osmotically migrates from one solution to another.

The pH electrode discovered at the turn of the century constitutes the first solid membrane, glass-type ion-selective electrode. Its properties, suggested mode of action, and related study of glass itself have been adequately covered in a number of texts (1, 2). The pH electrode or hydrogen electrode responds selectively to hydrogen ions in solution. Other glass-type electrodes which posess selective responses to cations other than hydrogen ions were developed later. These include sensors with response to both monovalent and divalent cations.

In early 1950 Wyllie and Patnode (3) discovered a generalized method of making solid ion-selective electrodes. They embedded the selective material in an inert solid membrane to form a heterogeneous membrane barrier. Although this technique represented a new concept in the construction of ion-selective electrodes, much progress in this field did not come until around 1966-1967 with the work of Ross and Frant on the ionized calcium electrode (4). Since then, heterogeneous ion-selective electrodes have been made for many ionic species (see Table 1). Inert materials containing different active substances were compressed into pellets or polycrystals and utilized as membrane barriers. Materials such as silicone rubber, polyvinyl chloride, polyethylene and later graphite were used as support media for different selective substances.

A major advance in the development of ion-selective electrode came in 1967 when Ross developed the first liquid membrane ion-selective electrode. He introduced an hydrophobic liquid in place of the solid material to form the barrier and incorporated the ion-selective substance into the liquid matrix (5). At about the same time Stefanac and Simon (6) were studying the ion-specific electrochemical behaviour of macrocyclic antibiotics (macrotetrolides) in membranous structures. In comparison studies involving potassium and sodium ions, they reported that antibiotic membranes posess greater selectivities than glass membranes.

This serves as further evidence for the observation that certain bilayer of lipid membranes when treated with macrotetrolides can act as highly selective electrodes. Consequently, liquid state electrodes incorporation Valinomycin, Gramicidin and Monensin as membrane substances were developed. These sensors show preferential selectivity to sodium and potassium ions.

Another recent addition to ion-selective electrode is the "Enzyme Electrode". Between 1971-1973 a number of papers was published in which it was stated that electrodes containing immobilised enzymes and related compounds could be used to monitor specific metabolites in solution. Specific enzymes, substrates and/or co-substrates form integral parts of membrane units where conventional enzymatic reactions occur with subsequent monitoring of changes in reactants and/or products. Although there are some technical problems in the construction of enzyme electrodes, their uses in the Clinical Chemistry Laboratory are becoming more apparent. The glucose-oxygen sensor(7) and the Kimble Blood Urea Nitrogen Analyzer®(8) are two examples of enzyme electrode systems commonly used in the Clinical Laboratory to determine levels of glucose and urea nitrogen, respectively, in biological fluids.

Also in recent years a number of gas-type electrodes have been introduced in the laboratory.

The basic concept is the same as for the solid membrane types. A hydrophobic gas-permeable plastic membrane separates the test and reference solutions. Electrodes for ammonia and sulphur dioxide gases are now available commerically (9).

An excellent review on ion-selective electrodes (10) has recently been published. Although the theory, principles, types and structure of the ion-selective electrode have been adequately dealt with, the applications of these sensors in the Clinical Chemistry Laboratory were not covered. Therefore, the purpose of this paper is to briefly discuss the basic principles of ion-selective electrodes and to demonstrate their applications in the laboratory.

CHAPTER 1

THEORY AND PRINCIPLES OF ION-SELECTIVE ELECTRODES (ISE)

The number and variety of ion-selective electrodes (ISE) is rapidly increasing with no apparent end in sight. It is possible at present to use these sensors to determine either by direct or indirect analysis, ionic concentrations of the species shown in Table 1, and those of aluminum, boron, chromium, cobalt, magnesium, mercury, nickel, phosphate, silver, sulphate and zinc. Before discussing the theory and principles of ISE, it seems appropriate to review the various types of ISE and the species sensed.

(A) ELECTRODE TYPES

Electrodes may be classified into two major groups: (1) Those composed of a solid barrier and (2) those composed of a hydrophobic liquid separating two hydrophilic solutions. They may be further categorized as summarized in Table 1 (11).

(1) SOLID STATE ELECTRODES

There are three kinds of solid state ISE. (a) The glass membrane type electrodes (b) doped or mixed crystal membrane type electrodes and (c) heterogeneous substance membrane type electrodes.

(a) GLASS ELECTRODES

The glass membrane type electrodes, originated

TABLE 1

Types of Ion-Selective Electrodes and Species Sensed.

LEGEND

Table 1 represents a general classification of known ion-selective electroqes. This Table is taken from reference 14 without the author's permission.

** ... Pressed pellets, impregnated polymer (PVC, PE, Silicone rubber), graphite.

*** ... Doped crystals, [a] ... Valinomycin, Gramicidin [b] ... Monensin.

TABLE 1

Types of Ion-Selective Electrodes and Species Sensed.

TYPES		SPECIES SENSED
SOLID STATE:	Glass	H^+, Na^+, K^+
	pH	H^+
	Crystals **	Cl^-, F^-, I^-, CN^-, SCN^-, SH^-
	Heterogeneous ***	Cd^{2+}, Cu^{2+}
LIQUID STATE:	Liquid Ion-exchange	Ca^{2+}, Cl^-, NO_3^-, K^+, ClO_4^-
		K^{+a}, Na^{+b}
OTHERS:	Gas	NH_3, SO_2, CO_2
	Immobilized biological reactants	glucose, urea, amino-acids.
	Combination	Cl^-, Na^+, F^-, redox

from the pH electrode (2, 7), are among the first generation ISE which are successfully used to determine ionic species in solution. Figure 1 shows the main features of the typical glass electrodes used in pH determination and as reference electrode. Experiments have shown that certain electrodes consisting of glass membrane would respond in a Nernstian manner to hydrogen ions in solutions. However, in alkaline solutions (high pH), the Nernstian relationship becomes invalid due to the sodium-ion response alkaline error. Eisenman and co-workers (12) showed that by adjusting the glass composition, it was possible to extend the sodium-ion repsonse to low pH and thus use the electrode to measure sodium ions selectively. Differential selectivity for such a glass membrane of 250:1 for Na^+ over K^+ have been reported (7). Similarly, glasses with high selectivity ratios of K^+ over Na^+ have been prepared (10). Based principally on the studies of Eisenman and co-workers with alumino-silicate glasses, a range of monovalent cation-responsive glass electrodes are available commercially. A summary of the literature on glass electrodes has been published by Ives and Janz (1). The structure of a typical sodium-ion glass electrode is also shown in Figure 1.

(b) DOPED (MIXED) CRYSTAL MEMBRANE ELECTRODES

This group of sensors represents electrodes consisting of solid phase membranes made of certain salt

FIGURE 1

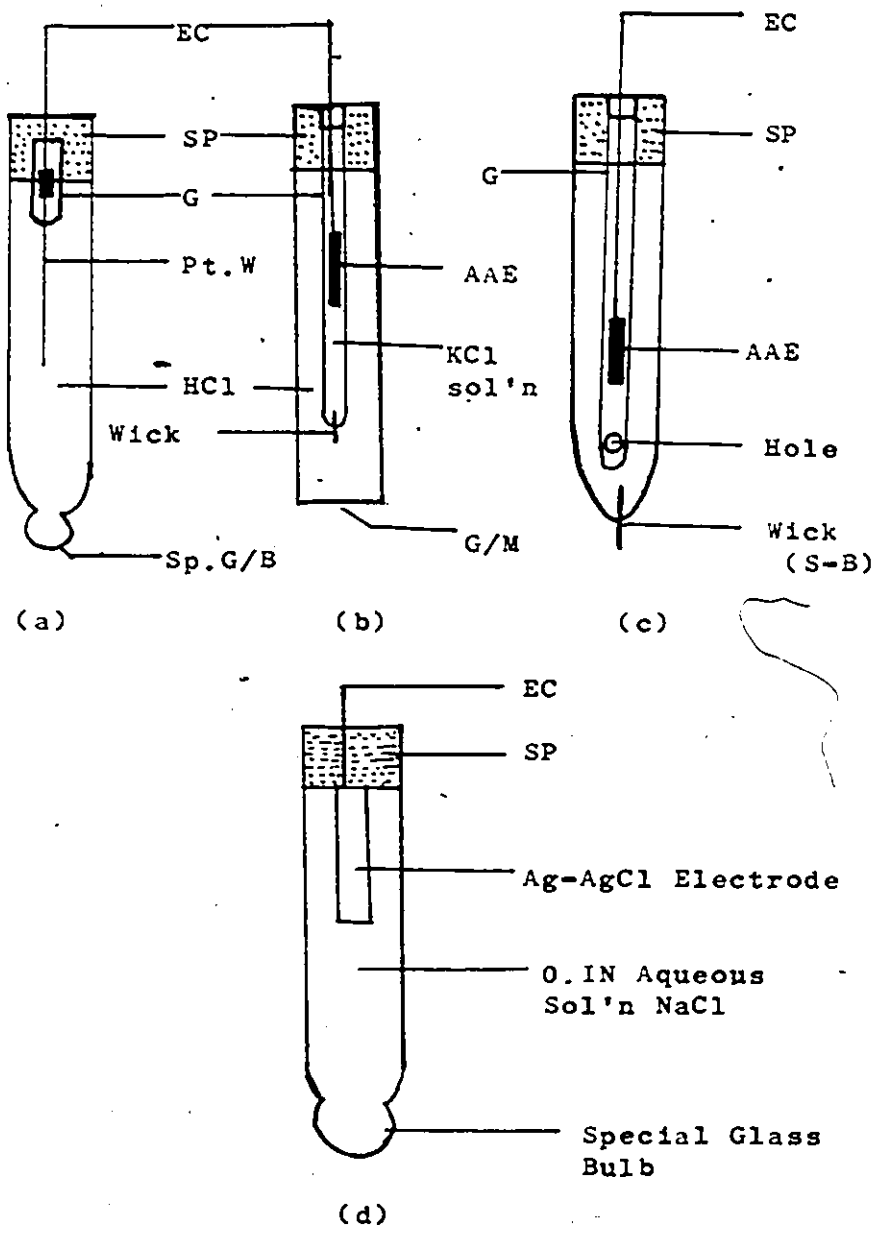
Structural Features of Typical Glass Membrane Electrodes.

LEGEND

Diagrams 1(a) and 1(b) represent glass electrodes used in the measurement of pH; 1(c) shows a typical reference electrode; 1(d) shows a sodium ion-selective electrode. EC - external connection; SP - space plug; Pt.W. - platinum wire; AAE - Ag/AgCl electrode; Sp. G/B - special glass bulb; HHE - Hg/Hg_2Cl_2 electrode; G - glass membrane.

FIGURE 1

Structural Features of Typical Glass Membrane Electrodes.



mixtures. Crystals such as LaF_3, Ag_2S and the silver halides are used directly as membranes or incorporated into inert matrices. Typical examples of this type of ISE are those used in the estimation of monovalent anions such as chloride, fluoride, cyanide, iodide and thiocyanate.

(c) HETEROGENEOUS SUBSTANCES MEMBRANE TYPE ELECTRODES

The essential feature or these electrodes is a solid membrane composed of a variety of heterogeneous materials. The nature and composition of the membrane depends on the ionic species to be sensed. Practically, there are two basic types; the general or combination type as shown in Figure 2. Usually they are self-contained sensors with built-in half-cell reference electrode. Others are of the simple type as shown in Figure 2, and require separate reference electrodes.

(2) LIQUID STATE ELECTRODES

Liquid state ISE are of two basic types; (a) those composed of an ion-exchange liquid membrane and (b) those with a neutral-carrier liquid membrane. Basically, the two types of electrodes are quite similar in structure, except that the mode of transport of ionic species across the membrane is different. The nature and composition of these electrode membranes will be discussed in more detail in CHAPTER 11. Typical examples of liquid ion-exchange ISE are those used in the estimation of ionized calcium, chloride, nitrate, sodium and potassium. Figure 2 shows a diagramatic

FIGURE 2

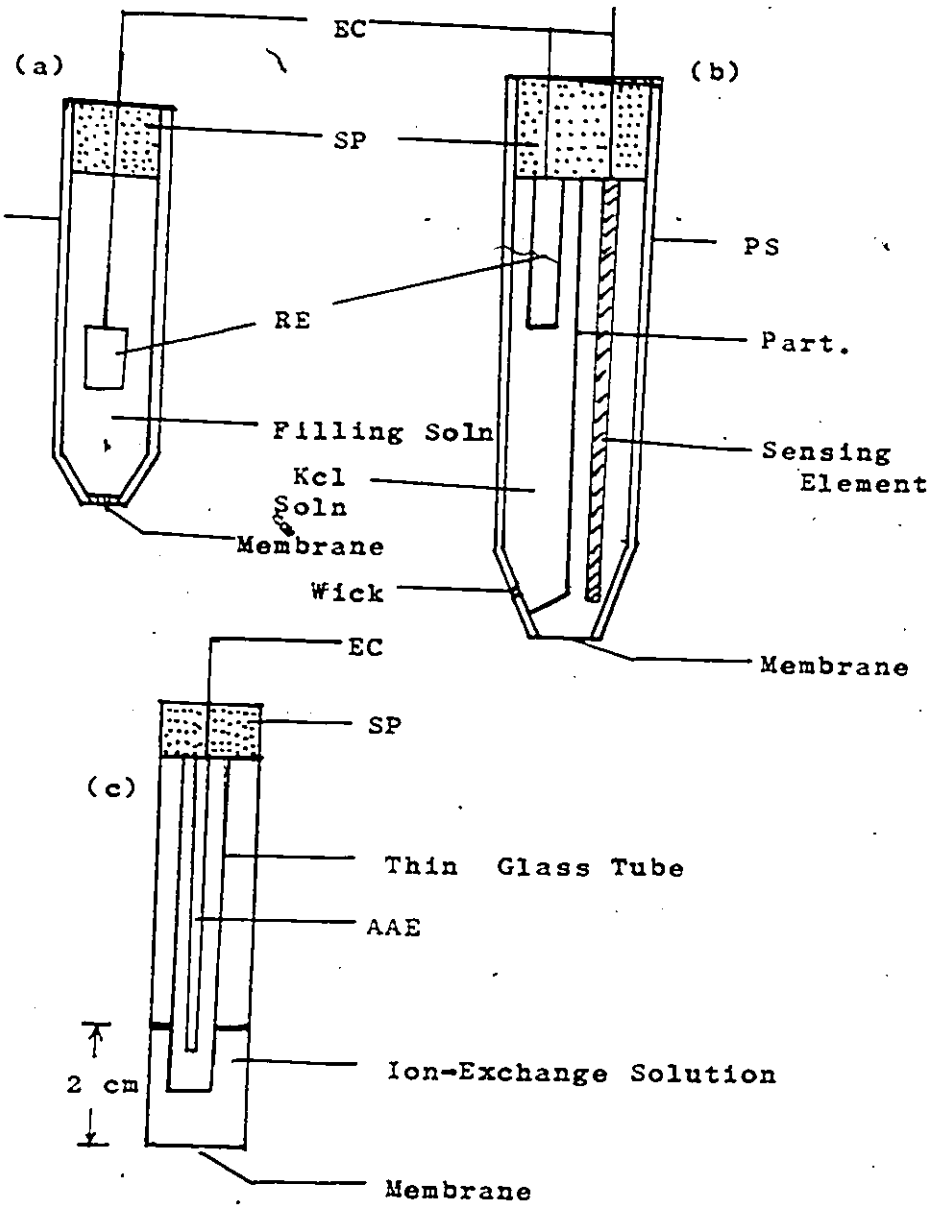
Structural Features of a Simple, a Combined and an Ioniszd Calcium Ion-Selective Electrodes.

LEGEND

(a) .. A simple electrode, (b) .. A general or combined electrode (self-contained with built-in half-cell reference electrode). PS: - plastic (fluorocarbon shell), AAE, EC, SP, as in Figure 1, part .. partition, (c) .. Structure of an ionized calcium electrode. EC, SP, AAE as for Figure 1. RE is the reference electrode.

FIGURE 2

Structural Features of a Simple, a Combined and Ionized Calcium Ion-Selective Electrodes.



representation of a typical liquid-membrane ion-selective electrode.

(3) OTHER TYPES OF ELECTRODES

In addition to the previously mentioned types of electrodes, there are a number of different varieties of ISE. Based mainly on the criterion of charge transport and selectivity, electrodes with selective responses to gases are available commercially. Examples of these sensors are ammonia, sulphur dioxide, and nitric oxide electrodes. They are usually of the general type shown in Figure 2 , in which a hydrophobic gas-permeable membrane separates the internal filling solution from the test solution. Another type of electrode meeting such criterion is the enzyme electrode. Biological reactants, usually enzymes, act as intermediary agents in electrode measurement systems. Their actions are generally highly selective and the end-products of their reactions are frequently simple ions for which selective electrodes already exist, e.g. urea electrode developed by Guilbault and Montalvo (13). Thirdly, hybrid types of ISE, commonly referred to as combination electrodes, are also available commercially. These are various combinations and/or modifications of liquid or solid state electrodes with built-in reference electrodes. For example, combination electrodes for estimation of chloride, fluoride, sodium and for the measurement of oxidation-reduction states are

marketed by Orion Research (14). These electrodes allow measurements to be made on very small sample sizes, on surfaces, filter paper and on confined spot test paper.

(B) ELECTRODE POTENTIAL AND RESPONSE

One of the fundamental requirements of any practical electrode is that its response at all times reflects the activity of the ion of interest in the solution to which the electrode is exposed (7). Thus the electrode potential is a direct measure of the ionic activity of the species sensed.

(1) ELECTRODE POTENTIAL

Empirically, the electrode potential of an ion-selective electrode is directly proportional to the logarithm of the activity (concentration) of the ionic species and is given by the Nernst Equation:

$$E_{ISE} = E^{\circ} + \frac{RT}{ZF} \ln a \quad \ldots\ldots\ldots \quad (1)$$

where E_{ISE} is the electrode potential, E° is the standard electrode potential, R and F are constants, Z is the valence of the ion, T is the absolute temperature and a is the activity of the ion. Theoretically, if the response is completely Nernstian, at 25°C a ten-fold change in concentration of a given univalent ionic species in solution will produce a change of 59.2mV (11). This theoretical prediction is very useful for calibration of ion-selective electrodes and as observed in most practical cases, only few ISE possess a true Nernstian relationship at desired concentration

ranges for a given ionic species. Most practical electrodes show near Nernstian relationship with respect to concentration.

(2) ELECTRODE RESPONSE

Although ion-selective electrodes are relatively specific in their responses to ionic species in solution, no one electrode posesses absolute specificity for a given ion over another. Consequently, a correction must be applied to the Nernst equation in cases of solutions with potentially interfering ions. Such a correction is afforded by the Nicolski type equation:

$$E_{ISE} = E^{o} + \frac{RT}{ZF} \ln a_1 + \sum_{J=1}^{n} K_{1J} \times a_j^{z/y} \quad \ldots\ldots\ldots \quad (2)$$

where Z is the charge on the primary ion including its sign, y is the charge on the interfering ion, a_1 and a_j are activities of primary and interfering ions, respectively, and K_{1J} is the selective coeficient. For two univalent cations this can be simplified as follows:

$$E_{ISE} = E^{o} + \frac{RT}{F} \ln (a_1 + a_2 \times K) \quad \ldots\ldots\ldots \quad (3)$$

where a_1 and a_2 are the activities of two univalent cations, K is the selectivity constant and the other parameters are as stated for equation 1.

The nature of the electrode response is also influenced by the membrane matrix materials. Studies on the relative sensitivities of glass electrode to various cations and mixtures of many univalent cations at constant pH, illustrate that solid ion-exchangers are

highly selective to univalent cations but considerable less so to divalent cations (15). This is attributed to the low mobility of divalent cations within the solid exchanger. Thus, as shown by Sandblom, Eisenman and Walker (16), the electrode potential of ion-exchange membrane, whether solid or liquid, at any time t, is given by the equation:

$$E_{ISE(t)} = \frac{RT}{Z_i F} \ln \frac{\sum_i U_i K_i a_i'}{\sum_i U_i K_i a_i''} - \int_1 - \int_2 \quad \ldots\ldots \quad (4)$$

where Z_i is the valency of i^{th} counter ionic species a_i' and a_i'' are their activities in solutions on each side of the membrane ($'$ = inside, $''$ = outside), U_i is the mobility within the membrane and K_i is a constant characteristic of the difference of standard potential in the membrane against water. $\int_1$ and $\int_2$ are the integrals across the thickness of the membrane ranging from 0 to diameter d and are particular characteristics of the liquid exchanger. Note the term "counter ion" is used here relative to the ions in or at the surface of the membrane matrix.

The potential response to a given ion (counter ion) is not only dependent on the activity and mobility, but also on the equilibrium constant of the ion exchange process occurring within and outside the membrane. Sandblom and co-workers (17) deduced the following expression for the potential of a liquid ion-exchange membrane for the special case of two counter ions and strong association between ion and site in the

membrane phase as:

$$E = \text{constant} - (1 - \tau)\ \frac{RT}{F}\ \text{In}\ A - \frac{RT}{F}\ \text{In}\ B \quad \ldots \quad (5)$$

where A and B are as follows:

$$A = \left(a_1 + \frac{u_2 + u_s}{u_1 + u_s}\ \frac{k_2}{k_1}\ a_2 \right) \quad \ldots\ldots\ldots\ldots\ldots \quad (6)$$

$$B = \left(a_1 + \frac{u_{2s} + k_2}{u_{1s} + k_1}\ \frac{K_1}{K_2}\ a_2 \right) \quad \ldots\ldots\ldots\ldots\ldots \quad (7)$$

and u_1, u_2, u_s, u_{1s}, u_{2s} are the mobilities in the membrane phase of anion 1, anion 2, the dissociated resin cation, the undissociated ion pair of resin cation and anion 2, respectively. k_1, k_2 are constants related to the standard chemical potentials of anion 1 and 2, respectively, K_1, K_2 are dissociation constants of ion pairs of the resin cation with anions 1 and 2, a_1 and a_2 are the activities of anions 1 and 2 and τ is as shown in equation 8:

$$\tau = \left[u_s \left(K_1 - u_{2s} - K_2\ u_{1s} \right) \right] \quad \ldots\ldots\ldots\ldots\ldots \quad (8)$$

Equations 5 - 8 are particularly useful when studying the selectivity ratios of ion-selective electrodes for both cations and anions.

Other factors which may influence tne response of ISE are: the presence of proteins and other constituents which can coat the surface of the membrane; the existence of the desired species in a form to which the electrode is insensitive; the ionic strength and pH of

the solution and the presence of "bound" water (water molecules trapped in the membrane). Thus protein-free filtrates, decomplexing or solubilizing agents, buffering agents and special solvents are often recommended for potentiometric determination of ionic concentrations with ISE.

(3) SELECTIVITY

Ion-selective electrodes as indicated earlier are not entirely specific for one particular ion and may respond to other ions in varying degrees of sensitivity. Thus, these sensors are said to have selectivity ratios or coefficients, K with respect to interfering ions. K is a measure of the electrode's ability to respond to one ion in preference to another - both being present in the same solution. In most cases manufacturers have determined selectivities for commercial electrodes for the common interfering ions, at least to a first approximation (14). However, care must be taken when using these selectivity coefficients because they are dependent upon experimental conditions. Generally, there are three different approaches used in the determination of selectivity coefficients (18). All three methods make use of the Nicolski relationship (see equation 3). In method 1 the electrode potential E_1 of a solution containing the primary univalent ion only is determined. According to the Nernst equation this potential is represented by the expression:

$$E_1 = E^o + \frac{RT}{F} \text{ In } a_1 \quad \ldots\ldots\ldots\ldots \quad (9)$$

Then the potential E_2 of a second solution containing the interfering ion only is determined under the same conditions. Taking the simplified case of two univalent cations, the electrode potential is given by equation 10 (previously stated as equation 3):

$$E_2 = E^o + \frac{RT}{F} \text{ In } (a_1 + K\, a_2) \quad \ldots\ldots\ldots \quad (10)$$

Substituting $a_1 = 0$ in equation 10

$$E_2 = E^o + \frac{RT}{F} \text{ In } K + \frac{RT}{F} \text{ In } a_2 \quad \ldots\ldots \quad (11)$$

Combining and rearranging equations 9 and 11, for $a_2 = a_1$ (equimolar concentrations of anions a_1 and a_2)

$$K = \exp \left(\frac{F}{RT} (E_1 - E_2) \right) \quad \ldots\ldots\ldots \quad (12)$$

Since E_1 and E_2 are measured and R, T and F are constants, K can be calculated.

A second approach makes use of similar conditions but in the second measurement the concentration of the interfering ion a_2 is chosen to give a potential $E_2 = E_1$. Since the primary ion is absent in experiment two, then combining equations 9 and 11, $K = \frac{a_1}{a_2}$. $\quad \ldots\ldots\ldots \quad (13)$

This method is very tedious and not widely used. The third approach combines methods 1 and 2. Unlike the previous two methods where only one ion pair is present in the test solution at any one time, both ion pairs are present in solution for the second measurement.

Consequently, by combining equation 9 and 11 and rearranging,

$$K = \frac{\left[a_1 - \exp\left(\frac{F}{RT}(E_1 - E_2)\right) \right] - a_1}{a_2} \quad \ldots\ldots\ldots (14)$$

a_1 and a_2 are activities of ionic species in the two measurements. They may or may not be equal. This method is usually the method of choice since conditions used are closer to those encountered in biological specimens. Light and Swartz made use of this method to evaluate selectivity of some common ISE (19).

Tables 2 and 3 show selectivity ratios obtained by the three different methods for a chloride ion-selective electrode in the presence of varying activities of bromide and iodide. Occasionally, method 3 is done graphically by plotting:

$$a_1 - \exp\left[\frac{F}{RT}(E_1 - E_2)\right] - a_1 \text{ against } a_2$$

This gives a straight line of intercept 0 and slope K.

(C) PRINCIPLES OF OPERATION

The general principles of operation of all ISE are based on direct or indirect potentiometric measurements in relationship to ionic activities. The types of measurements made and techniques used are varied and dependent on the nature and quantity of ionic species sensed, the reaction system and the kinds of equipment available.

(1) TYPES OF MEASUREMENTS

Ion-selective electrode measurements are made in

TABLE 2

Selectivity Coefficient K of the Chloride Selective Electrode For Iodide Ion.

LEGEND

These data were taken from reference 18 without the author's permission. Note the inter-and intra-method variations in values obtained for the selectivity coefficient, K.

TABLE 2

Selectivity Coefficient K of the Chloride Selective Electrode for Iodide Ion.

Cl^-	Method 1		Method 11			Method 111		
	a_{Cl}	K	a_{Cl}	a_I	K	a_{Cl}	a_I	K
100.0	75.5	17.1	75.5	2.83	26.7	75.5	20 - 40	23.9
10.1	8.99	16.3	16.6	0.964	17.6	9.0	3.6 - 8	20.7
1.0	0.964	13.3	9.99	0.612	17.3	2.0	.36 - 1.8	14.8
0.1	0.099	4.4	0.469	0.009	4.8	0.20	.0038-0.04	6.0

Activities and concentrations are expressed as millimolar (mM)

a_X are the activities of the corresponding anions.

TABLE 3

Selectivity Coefficient, K of the Chloride Electrode for Bromide Ion.

LEGEND

These date were taken from reference 18 without the author's permission. Note again the inter- and intra-method variations in the values obtained for the selectivity coefficient, K.

TABLE 3

Selectivity Coefficient K of the Chloride Electrode for Bromide Ion.

Method 1			Method 11			Method 111		
Cl^-	a_{Cl}	K	a_{Cl}	a_{Br}	K	a_{Cl}	a_{Br}	K
100.0	75.5	2.70	75.5	20.2	3.73	75.5	20 - 40	3.42
10.0	8.9	2.88	29.6	8.99	3.20	17.2	4.0-17	3.47
1.0	0.964	2.7	2.88	0.964	2.99	1.86	0.37-1.8	2.95
0.1	0.099	1.72	0.202	0.099	2.04	0.19	.038-0.19	2.58

Activities and concentrations are expressed in millimolar (mM)

a_x Represent the activities of the respective anions.

determining rates of reactions, equilibrium constants, concentration gradients and transients. Usually, the amounts of analytes present in the system are too small to be accurately assayed by other coventional methods, or the analytes exist in the presence of undesired ions. Therefore, ion-selective electrodes, because of their relatively high sensitivities and selectivities, are the methods of choice in the above cases.

(2) TECHNIQUES USED FOR QUANTITATION

There are two basic approaches used in the quantitative determination of an ionic species by ISE: (a) direct analyses where the concentration is read from a standard curve or a direct read out meter, (b) indirect analyses which involve known addition, known subtraction and titration methods.

(a) DIRECT ANALYSES

Electrode potentials are observed in standardizing solutions prepared by serial dilution. The potentials are then plotted on semi-log graph paper to give a straight line and the concentrations of the unknown solutions read from the standard curve. An alternative approach is to read the concentrations directly from a specific-ion meter following calibration with one or two standardizing solutions (20).

(b) INDIRECT ANALYSES

Indirect analyses may be subdivided into three classes. These are known addition / subtraction, titration and graphical procedures.

(i) KNOWN ADDITION/SUBTRACTION PROCEDURES

In the known addition method, a known amount of the species being measured is added to a known volume of the sample (usually in the ratio of 1:10, respectively) and the resulting change in potential is observed. The original sample concentration is then computed using a known addition table relating change in potential to concentration (9) or, read from a direct read out meter. Known additions allow samples with variable ionic strength to be measured, even in the presence of complexing agents. No calibration curve is required for known addition or subtraction method. Therefore, it is quicker than methods involving standardization. The known subtraction procedure is similar to the known addition method except that the standard solution which is added, contains a complexing or precipitating agent for the sample species. The former method is used to analyze sample species which are unstable, thus making the preparation and storage of standardizing solution difficult.

(ii) TITRATION PROCEDURES

Titration greatly increases the number of species which can be measured by ISE. There are three basic types of titrations (17), R, S and T titrations (see Figure 3). R titrations are called indicator titrations. The electrode actually senses the level of the reagent (R) species which has been added to the sample

FIGURE 3

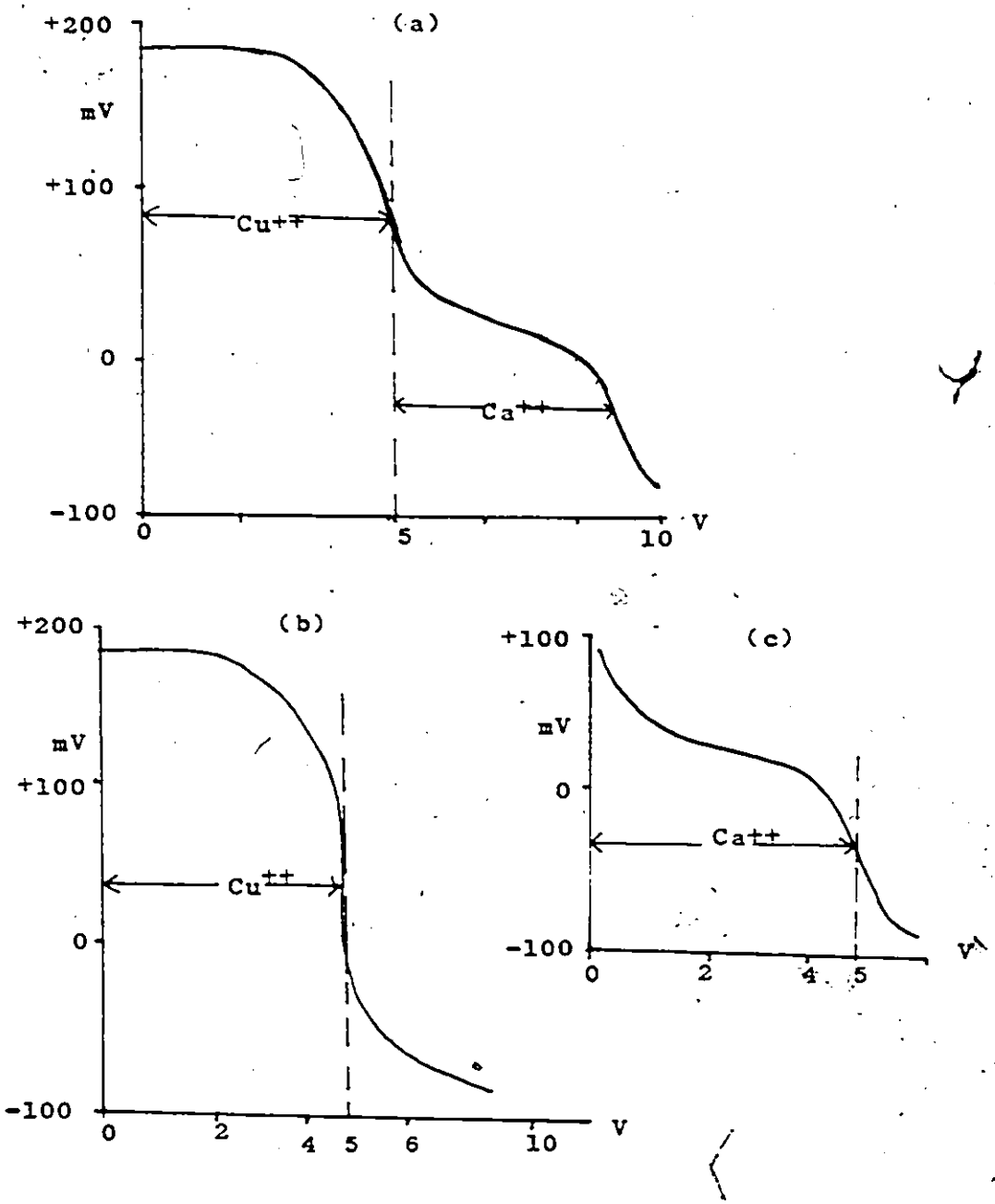
Typical Titration Curves for Ion-Selective Electrodes.

LEGEND

In Figure 3 typical titration curves for the Orion copper (+2) 94-29 electrode are shown (14). 3(a) shows the titration of 100 ml of sample 10^{-3} M Cu++ and 10^{-3} M Ca++ (note the two end-point breaks). 3(b) is a typical S titration curve for the volume of sample containing 10^{-3} M Cu++. 3(c) is a typical R titration curve for 100 ml-sample containing 10^{-3} M Ca++ (Cu-EDTA is added as indicator). In all three cases Na_4EDTA titrant is added to the sample. V represents the volume of the titrant added.

FIGURE 3

Typical Titration Curves for Ion-Selective Electrodes.



before titration (17). These titrations are useful in determining metal ions for which there is no electrode or T titration available. Examples include titrations for nickel, zinc and cobalt. In S titrations the species sensed by the electrode is the sample (S) species. As the titration proceeds, the level of S is lowered due to the complexing or precipitation of S by the titrant. S titrations are used when the species to the sensed must be analysed with greater accuracy than can be obtained by direct measurement. In T titrations, the electrode senses the titrant (T) species. Examples of this type of measurement include the titration of lead with sulphate and of aluminum with fluoride electrodes, respectively.

(iii) GRAPHICAL METHODS

Graphical methods using the known addition/ subtraction procedures are represented by the Gran's plots (14). The observed electrode potentials are converted to a series of "apparent concentrations" and plotted against volume of unknown additions and subtractions made. A straight line is fitted to the points and extrapolated back to the horizontal axis to obtain the sample concentration. Alternatively, electrode potentials can be plotted against volume directly without calculation on a special graph paper (Gran's plot paper) developed by Orion, and concentration determined by extrapolation as before. Both T and S

titrations can also be performed on Gran's plot paper. Graphical methods are usually quicker than the conventional titration and known addition/subtraction methods. The former also produce more accurate results.

CHAPTER II

MATERIALS USED IN MEMBRANE BARRIERS

The term "membrane" as applied to ISE represents a thin section of electrically conducting material separating two solutions and across which a potential develops. Various selective substances are used as membrane components. The incorporation of these materials into membrane barriers of ISE is as important as the development of the electrodes themselves. Over the past two decades a great deal of time and effort has been spent on the development of membrane barriers with high ion selectivities, resulting in the ISE as it is known today. Studies by Eisenman and co-workers (12), Wyllie and Patnode (3), Ross and Frant (4), Ross (5), Mueller and Rudin (11), Stefanec and Simon (6), Baum and Ward (11), Buck (11), Mohen and Rechnitz (11), Gough and Andrade (21), and Llenado and Rechnitz (22) illustrate the wide variety of substances employed in the fabrication of such membraneous structures. These substances include liquids, inorganic and organic compounds (particularly polymers), glass, clay, biological reactants and antibiotics. Although time will not permit a detailed discussion of the various kinds of materials employed as components of membrane barriers, because of their importance in the development of ISE, a brief review on each class of

of membrane is given below.

(A) CLAY MEMBRANES

Clay membranes are prepared by carefull regulated heating of specially selected clays. By regulation of the doping levels, the clay mixture carries the necessary ions which control the absorption of other ions to the membrane and give rise to the selectivity of the membrane. These membranes are difficult to prepare, have high electrical resistance and are easily broken. Consequently, their usage in electrode are limited. Studies on clay membrane electrodes were pioneered by Marshall and Marshall and Bergman between 1939-1942 (23). Earlier membranes consist of thin plates formed from natural single crystals of chabazite, apophyllite (from zeolites), montmorillinite and bentnonite (from clay). Some of the problems encountered with these earlier types of clay were that the colloid suspension used became dried at high temperature (490°C) and that sodium ions interfered with the determination of potassium. This led to the development of special clays by Marshall in 1948 (21).

(B) GLASS MEMBRANES

Glasses used as membranes in ion-selective electrodes, vary considerable with respect to chemical formulae. However, the basic components are similar. Generally they have three components in common. These are SiO_2, an alkaline metal oxide and the oxide of bi- or trivalent metal. A typical glass which can be used in the

hydrogen-ion selective electrode (pH electrode) might have the following composition: SiO_2, 65%; Li_2O, 25%; Na_2O, 1%; BaO, 7%; La_2O_3, 2% (percentages represent mole %). Such glass is considered as a lithia glass. By modifying the formulation of the glass mixtures, Eisenman and co-workers developed glasses that are selective to a particular ion (12). For example, a sodium alumino-silicate glass of the following composition (mole%): Na_2O, 11%; Al_2O_3, 18%; SiO_2, 71%; have been reported to have a differential sensitivity of 250:1 for Na^+ over K^+ (12). Further studies have indicated that similar recipes can be developed to produce potassium selective glass. Glass of the following composition (mole %): Na_2O, 27%; Al_2O_3, 4 or 6%; SiO_2, 67 or 69%, have been known to be highly selective for K^+ (10).

Lithia glasses are used in preference to soda-lime glass in pH electrodes in attempt to overcome the sodium ion alkaline pH error discussed in CHAPTER 1. Lithia glasses also have the added advantage of requiring lower fusion temperatures.

(C) LIQUID MEMBRANES

(1) IMMOBILIZED LIQUID MEMBRANES

These are membranes which consist of a liquid ion-exchange resin held by an inert support. The utilization of liquid membranes in ISE is based on the mobility of their exchange sites. Usually these sites are of molecular sizes and immobilization can be carried out

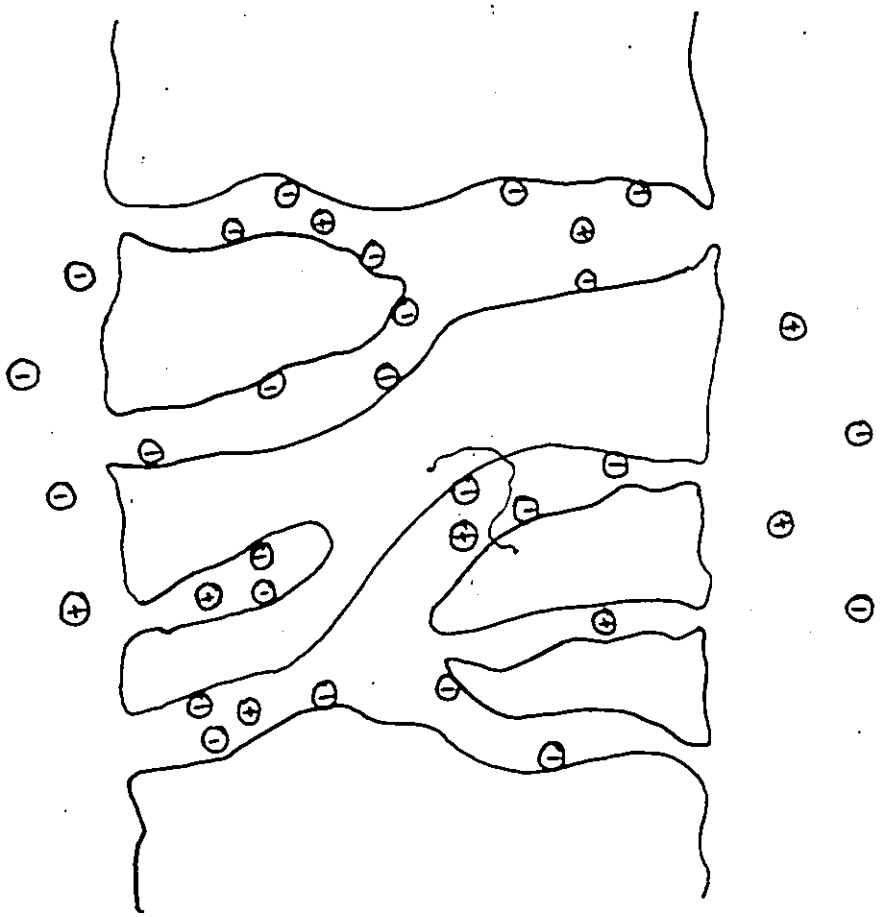
in a bulk matrix. This helps to minimize irregularities in membrane function due to poor liquid-liquid interface, stirring effects and pressure differentials. In 1943 Sollner claimed to have developed the first anion selective electrode utilizing an immobilized liquid membrane (24). Collodion was specially treated in protamine sulphate and used as membrane in an electrode which responds selectively to certain anions. By modifying the treatment conditions he was able to demonstrate that collodion can also be used in cation selective electrodes. Collodion is a mixture of pyroxylin in alcohol and ether. It represents an incomplete nitration of cellulose and is capable of supporting high ionic charge densities, thus making it highly selective. Another advantage is that the collodion membrane has an adjustable electrical resistance and like other inert support media, it does not enter into the selectivity of the membrane. It merely serves as support for ion groups lining the pore walls. However, there is one basic requirement of all such support media. They ought to be permeable to microscopic charge carriers. These membranes are generally referred to as permselective membranes. Figure 4 is a diagramatic representation of a perm-selective membrane redrawn from reference (21).

(2) LIQUID ION-EXCHANGE MEMBRANES

Liquid ion-exchange membranes function in a manner

FIGURE 4

Diagramatic Representation of a Perm-Selective Membrane.



⊖ Co-Ions

⊙ Fixed negative charges

⊕ Counter ions

analogous to collodion type membranes. Ion groups whose concentrations are to be measured are attached to suitable organic molecules of molecular weights between 300 and 600. The resulting compounds are dissolved in organic solvents and the solutions so obtained are used, in a thin layer, to separate two aqueous solutions of different ionic concentrations (reference and test solutions). Solvents used are usually those with low dielectric constants and are immiscible with water: eg. benzene, xylene, nitrobenzene (7) toluene, kerosine (paraffin) and higher alchols (10). A wide variety of organic compounds is employed as ion-group receptors. These include secondary and teritary amines, quaternary ammonium compounds and various organic acid compounds. For example, calcium stearate was used by Tendelno and Van der Voort in 1960 to detect ionized calcium (25), calcium oxalate by Cloos and Fripiat in 1961 to detect ionized calcium (26) and trialkylmethyl-lauryl amine by Sollner (24) and Hollos-Hokosinyl in 1961 to detect chloride and thiocyanide (27). The ion-group receptors or transport molecules carry the selective ion across the boundary while the organic solvent provides the barrier preventing mixing of the reference and test solutions.

(D) HETEROGENEOUS MEMBRANES

Heterogeneous membranes are usually referred to as non-collodion perm-selective membranes. They are divided

into two groups depending on their components. Those whose active ingredients are principally ion-exchange resins and those whose active ingredients are mainly salts and mixed crystals.

(1) THE HETEROGENEOUS ION-EXCHANGE MEMBRANES

Heterogeneous membranes are similar to liquid membranes. Their basic structures are almost identical except that unlike liquid membranes, heterogeneous membranes consist of solid matrices. Particles of ion-exchange resins are embedded in an inert binder which serves as a framework to support the resin between the solutions. Occasionally, membranes are produced in which the ion-exchange resins are subjected to polymerization in a membrane form. Polyethylene, polypropylene, polystyrene and polyvinyl chloride are among the common polymers used as support for ion-exchange sites in heterogeneous membranes. Silicone rubber and graphite have also been employed (11). Again, the constraint on these substances is that they are permeable to small charged particles.

(2) THE MIXED CRYSTAL MEMBRANES

Certain crystals are used as membrane phase in ISE. They may be used directly as membranes or may be incorporated into an inert matrix. Crystals such as LaF_3, Ag_2S and AgX (X = halide) are used as membranes in ISE. For example, the active element in the sulphide-ion-selective electrode is polycrystalline Ag_2S. Mixed

crystals derived from mixtures of Ag_2S with CuS, PbS or CdS were also used as membrane components in a number of ISE. The actual mode of action of these membranes is not as well understood as for glass or liquid membranes. One theory assumes rapid, reversable ion-exchange at the membrane interface with mobile defects within the membrane crystal, thus facilitating transport across the membrane. Evidence in support of this theory comes from the work of Hseu and Rechnitz (28). They have concluded that the transport of silver ions in the Ag_2S membrane of the sulphide-ion-selective electrode forms the principle of operation. It is well accepted that adsorption at the membrane surface is the primary step in the mechanism of ion-exchange. The overall results of ion adsorption, membrane effects and transport can be summarized by the following equations:

$$Ag_2S \rightleftharpoons 2Ag^+ + S^{2-} \quad \ldots\ldots\ldots\ldots \quad (15)$$

$$M^{2+} + S^{2-} \rightleftharpoons MS \quad \ldots\ldots\ldots\ldots \quad (16)$$

Charge is transported by the movement of Ag^+ ions and the electrode potential is determined by the availabiltiy of sulphide (S^{2-}) ions. The apparent applicability of the above system in titration methods involving ISE is obvious. As Rechnitz and Kummer *et al.* (29, 30) have indicated it is also possible to extend the concept of mixed crystal membranes to other two and three-component crystal mixtures besides sulphides. A mixture of Ag_2S, 32%; PbS, 31%; $PbSO_4$, 32%; CuS, 5% forms

the basis of the sulphate-ion-selective electrode. Similarly, a calcium-ion response electrode used by Perkin Elmer Corporation is made from a mixture of CaF_2 and LaF_3 (10).

(E) ENZYME AND RELATED SUBSTANCES MEMBRANES

Enzymes, coenzymes and substrates are held within membrane units, which in turn are used in ion-selective electrode systems. The basic functional concept of the enzyme system is the continous, instantaneous, electrochemical monitoring of enzyme-catalyzed reactions in which a substrate, coenzyme or inhibitor is converted into a product by means of an enzyme. By varying the relative concentration of the reactants, one can obtain analytical techniques in which the reaction rates or equilibrium concentrations are proportional to the limiting components.

The method of immobilization depends on the particular enzyme electrode system. In some cases the enzyme is trapped within synthetic hydrophobic gel, cross-linked to the membrane, chemically bound to the membrane and other surfaces or copolymerized with other proteins. In other cases the enzyme is physically trapped within the membrane. Basic and acidic groups may also be polymerized in the supporting matrix in which the enzyme is immobilized so that pH in the immediate vicinity of the enzyme is optimum while that of the bulk solution is different. For further

discussion on this topic, the reader is directed to the excellent review of Rechnitz and co-workers (28). Equations for some enzyme electrode systems in which the enzyme or substrate has been physically immobilized in the vicinity or bonded to the sensor are given below:

(1) GLUCOSE OXIDASE IMMOBILIZED SYSTEM

$$\text{Glucose} + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} \text{Gluconic acid} + H_2O_2 \quad \ldots\ (17)$$

In the early system of Clark and Lyons, glucose is held between cuprophane membrane and the consumption of oxygen is determined by means of an oxygen sensitive electrode (7). More recently, glucose oxidase is held between layers of dialysis paper and an oxidant or catalase is added to the reactant mixture (31):

(a) $$\text{glucose} + \underset{\text{(oxidant)}}{\text{quinone}} \longrightarrow \text{gluconic acid} + \text{quinone-}H_2 \quad \ldots\ (18)$$

(b) $$\text{glucose} + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} \text{gluconic acid} + H_2O_2 \quad \ldots\ (19)$$

$$2H_2O_2 + \text{catalase} \longrightarrow 2H_2O + O_2 \quad \ldots\ (20)$$

(2) IMMOBILIZED LACTATE DEHYDROGENASE (LDH)

$$\text{Lactate} + 2Fe(CN)_6^{3-} \xrightarrow{\text{LDH}} \text{Pyruvate} + 2H^+ + 2Fe(CN)_6^{4-} \quad \ldots\ (21)$$

$$2Fe(CN)_6^{4-} + Pt \longrightarrow 2Fe(CN)_6^{3-} + 2e^- \quad \ldots\ (22)$$

The lactate dehydrogenase is held between dialysis membranes and the ferrocyanide produced from the conversion of lactate to pyruvate is oxidized at the surface of a platinum wire electrode. A standard calomel electrode is used as reference.

(3) IMMOBILIZED UREASE SYSTEM

$$\text{Urea} + 2H_2O \xrightarrow{\text{urease}} NH_3 + NH_4^+ + HCO_3^- \quad \ldots\ldots \quad (23)$$

In the original urea transducer of Guilbault and Montalvo, urease is immobilized in a thin layer of acrylamide gel which is held over the surface of a cation electrode by a cellophane film (13). This system has been said to be susceptible to interference from sodium and potassium ions. Removal of these interfering cations by pretreating the specimen with ion-exchange resin are recommended with the above system. More recently a similar system used in conjunction with ammonia gas electrode has been developed (8). This system is said to be free of interference from sodium and potassium ions.

(4) IMMOBILIZED D- AND L-AMINO ACID OXIDASES

$$\underset{\text{(L-form)}}{NH_3^+\text{-R-COO}^-} + H_2O + O_2 \xrightarrow{\text{L-AAO}} O = \text{R-COO}^- + H_2O_2 + NH_4^- \quad \ldots\ldots\ldots \quad (24)$$

$$2H_2O_2 + \text{Catalase} \longrightarrow 2H_2O + O_2 \quad \ldots\ldots\ldots \quad (25)$$

Catalase is added to prevent further oxidation of the keto acid produced.

$$\underset{\text{(D-form)}}{^-OOC\text{-R-}NH_3^+} + O_2 \xrightarrow{\text{D-AAO}} {}^-OOC\text{-R} + NH_4^+ + CO_2 \quad \ldots \quad (26)$$

In both systems the enzymes are immobilized at the tip of commercially available cation electrodes. The general stabilities of these systems were tested and found to be satisfactory for use in the clinical laboratory.

(5) OTHER ENZYMES

Two other enzyme electrode systems worth mentioning are the glutamine and β-glucosidase systems:

(a) Glutaminase System:

$$\text{Glutamine} + H^+ \longrightarrow \text{Glutamic acid} + NH_4^+ \quad .. \quad (27)$$

This system can be used in conjunction with the ammonia electrode. Note that the system is pH dependent. Therefore, pH adjusters are necessary.

(b) β-Glucosidase System:

$$\text{Amygdalin} \longrightarrow 2\ \text{Glucose} + \text{Benzaldehyde} + HCN \quad .. \quad (28)$$

The β-glucosidase is immobilized in acrylamide gel which forms part of the membrane unit of a cyanide ion-selective electrode (21). The principle is that of the solid state electrode, already discussed in CHAPTER 1.

(F) MACROCYCLIC ANTIBIOTIC MEMBRANES

Certain antibiotics are known to be highly selective for alkali metal cations (32). Valinomycin and Actin are two such antibiotics associated with potassium ions in preference to sodium ions. When suspended in various organic solvents (nujol/2-octanol, nitro- and chlorobenzenes, chloroform, diphenylether) and incorporated into liquid membrane, these antibiotics give rise to electrodes with considerable high selectivity for potassium ions. Comparison studies have shown that electrochemical cells with macrotetrolide (actin homolog) membranes possess superior selectivity

for K^+ than glass electrodes(6). Frant and Ross (4) supported this observation when they demonstrated a selectivity ratio of 10,000:1 (K^+/Na^+) with a valinomycin electrode. Nonactin homologs used in ISE are excellent complexing agents for potassium ions, but they possess much lower selectivity ratios than valinomycin and nonactin. Nonactin homologs are generally referred to as macrotetrolides.

CHAPTER III

APPLICATIONS IN THE CLINICAL CHEMISTRY LABORATORY

Until recently the use of ISE in the clinical laboratory has been limited to just a few cases such as in the determination of blood pH and gases. Although ISE offer certain advantages over corresponding colorimetric procedures, the full usefulness of these sensors had not been realized in the clinical laboratory. There are two main reasons for this. Automated sampling and reagent-mixing systems had not been modified for use with these sensors. Seconaly, because of technical difficulties, ISE methods are labelled impractical for routine clinical use and therefore employed mainly as research tools. However, in the last ten to twelve years there has been a general increase in the utilization of ISE systems routinely in the clinical laboratory. Commercially available ISE systems for direct measurement of blood, plasma and urine ammonia, ionized calcium, glucose, urea, sodium, potassium and chlordie are now used routinely in several laboratories.

(A) DETERMINATION OF BLOOD OR URINE AMMONIA

(1) CLINICAL SIGNIFICANCE

The estimation of blood or plasma ammonia has been used as an aid in the diagnosis of hepatic coma or impending hepatic coma in patients with severe liver

dysfunction caused by cirrhosis and various types of neoplasms. More recently the detection of hyperammonaemia has been used to pinpoint genetic defects in the enzymes of the urea cycle in neonates and young children. Since 1963, when first described by Reye and co-workers, there has been considerable interest in the diagnosis of acute hepatopathic-enecephalopathic diseases of children, now known as Reye's Syndrome (33). Over the years several methods have been developed and used in the determination of plasma ammonia. Diffusion methods (34), ion-exchange resin methods (35) and enzymatic methods (36) are often unsatisfactory. The analysis of ammonia by ISE represents a new approach and was first introduced by Orion in 1973 (9).

(2) QUANTITATIVE ESTIMATION OF AMMONIA BY ISE

(a) THEORY OF OPERATION

Ammonia liberated from a sample following alkalinization, diffuses through a hydrophobic gas permeable membrane into an internal filling solution consisting of aqueous ammonium chloride until the partial pressure is the same on both sides of the membrane. The level of ammonia gas in the sample is measured by monitoring pH changes in the internal filling solution, caused by hydrolysis of a small portion of the dissolved gas:

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \quad \ldots\ldots\ldots\ldots \quad (29)$$

According to the law of mass action:

$$\frac{[NH_4^+][OH^-]}{[NH_3]} = \text{constant} \quad \ldots\ldots\ldots\ldots \quad (30)$$

If the ammonium ion concentration in the internal solution is considered constant, then:

$$\left[OH^-\right] = \left[NH_3\right] \times \text{constant} \quad \ldots\ldots\ldots\ldots\ldots (31)$$

that is, ammonia gas in the internal filling solution varies directly as the hydroxide ions. The electrode potential is given by the Nernst equation:

$$E = E^\circ - \frac{2.303RT}{F} \log \left[OH^-\right] \quad \ldots\ldots\ldots\ldots\ldots (32)$$

with the symbols E, E°, R, T, and F as before (see equation 1). Since OH^- is proportional to the ammonia concentration, the electrode response can be represented as:

$$E = E^\circ - \frac{2.303RT}{F} \log \left[NH_3\right] \quad \ldots\ldots\ldots\ldots\ldots (33)$$

Normally it is more convenient to measure changes in hydrogen ions rather than changes in hydroxyl ions. Thus, equation 32 can be rewritten using the relationship $\left[H^+\right]\left[OH^-\right] = 10^{-14}$ as:

$$E = E^\circ = \frac{2.303RT}{F} \log (K \times 10^{-14}/H^+) \quad \ldots\ldots\ldots (34)$$

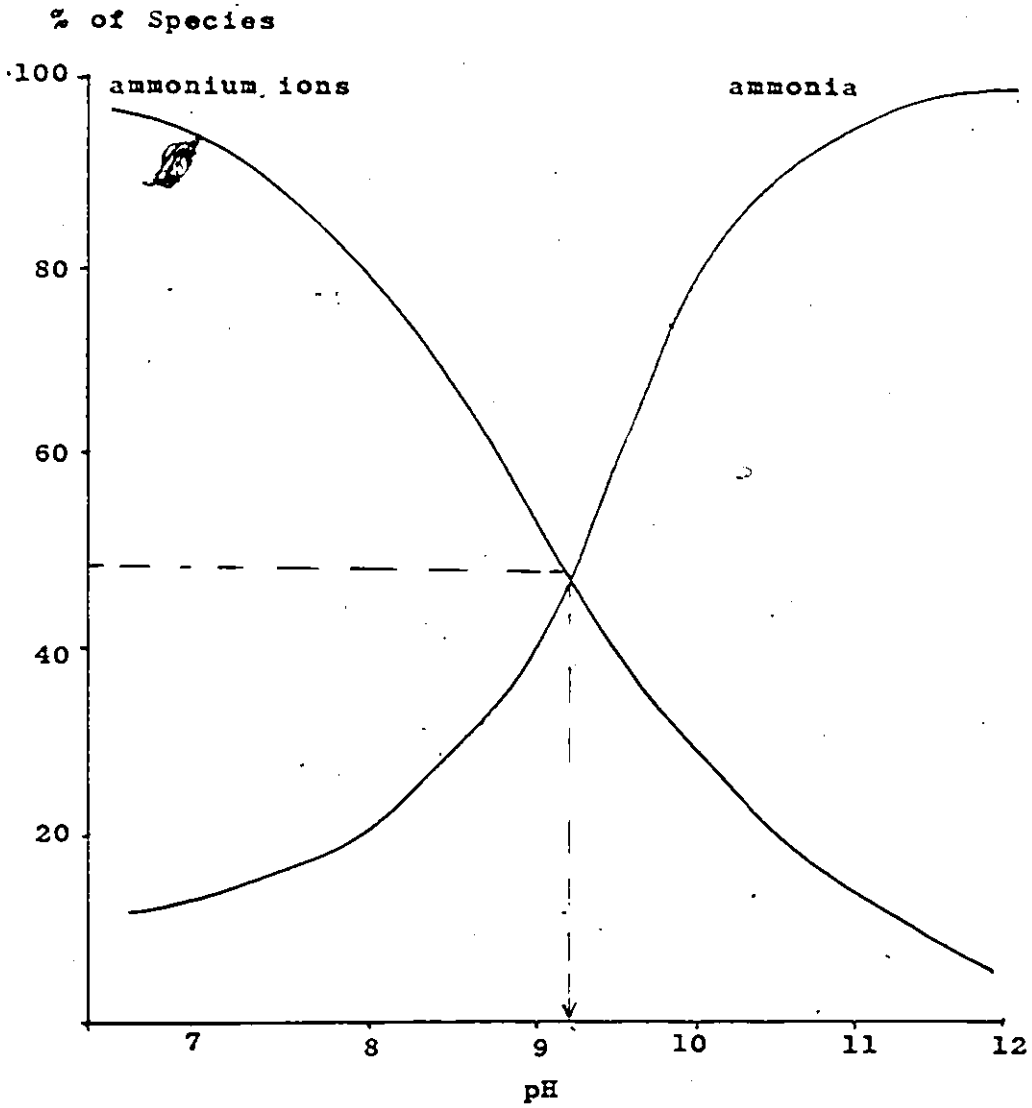
Since pH is defined as $-\log H^+$, then equation 34 becomes:

$$E = E^\circ - \frac{2.303RT}{F}(-pK + pH - 14) \quad \ldots\ldots\ldots\ldots (35)$$

That is, the relative amount of ammonium ion and ammonia present in the internal filling solution and, hence the electrode potential, is a function of the pH of the solution. Figure 5 shows the fraction

FIGURE 5

Fraction of Ammonia and Ammonium Ion as a Function of pH for the Ammonia-Selective Electrode.



of ammonia and ammonium ion as a function of pH for the Orion ammonia electrode (series 95-10). About 50% of the ammonia is in the form of ammonium ion at pH 9.2. The concentration of ammonia in the filling solution is also dependent on the partial pressure of the ammonia gas. Henry's law of partial pressure relates the ammonia concentration to the partial pressure:

$$P_{NH_3} = K_L \cdot [NH_3] \quad \ldots\ldots\ldots\ldots\ldots \quad (36)$$

P_{NH_3} is the partial pressure of ammonia, K_L is the Henry's law constant and $[NH_3]$ is the concentration of ammonia. K_L as well as pK vary with temperature and ionic strength. Therefore, in practice, standards and unknowns should contain the same level of dissolved species and be at the same temperature.

(3) METHODS

The methods of estimation of ammonia in serum plasma, whole blood and urine depend on the type of electrode system used. However, direct measurements using calibration curves appear to be the method of choice. Direct analyses can be done manually or by automation.

(a) MANUAL PROCEDURES

The electrode is placed in standard solutions prepared by serial dilution, an aliquot of strong alkali (11MNaOH) added to liberate ammonia and the electrode potential recorded after one to three minutes. Millivolts readings are then plotted against concentration

on semi-log graph paper to produce a straight line as shown in Figure 6. Unknowns are treated similarly and their concentrations read from the calibration curve. Usually, 1.0 ml of sample added to 100 ml of ammonia-free distilled water is the required amount. Recently Proelss and Wright (37) have developed a direct method for estimating ammonia in perchlorate supernate. Blood proteins are precipated with 8% perchlorate and 3.0 ml of the supernate used for direct measurement of ammonia by ISE. The use of perchlorate supernate eleminates errors due to ammonia liberated from proteins or from protein interactions with the hydrophobic membrane. It also makes it possible to carry out assays on small volumes. However, in order to maintain the electrode stability, they found it necessary to modify the manufacturer's internal filling solution so that the osmolality is approximately the same as that of the filtrate. Thus an internal filling solution consisting of 0.1 M NH_4Cl, 0.25M Na_2SO_4 (total osmolality of about 700 mosmol/kg) was used instead of the manufacturer's (average osmolality of 80 mosmol/kg). This method forms the basis of the automated version discussed in the APPENDIX under user's experience with two ISE systems. All attempts by us to use this method were unsuccessful, even when the electrode was assembled and used exactly as stated by Proelss *et al.* (37).

Blood ammonia is also determined by the known

FIGURE 6

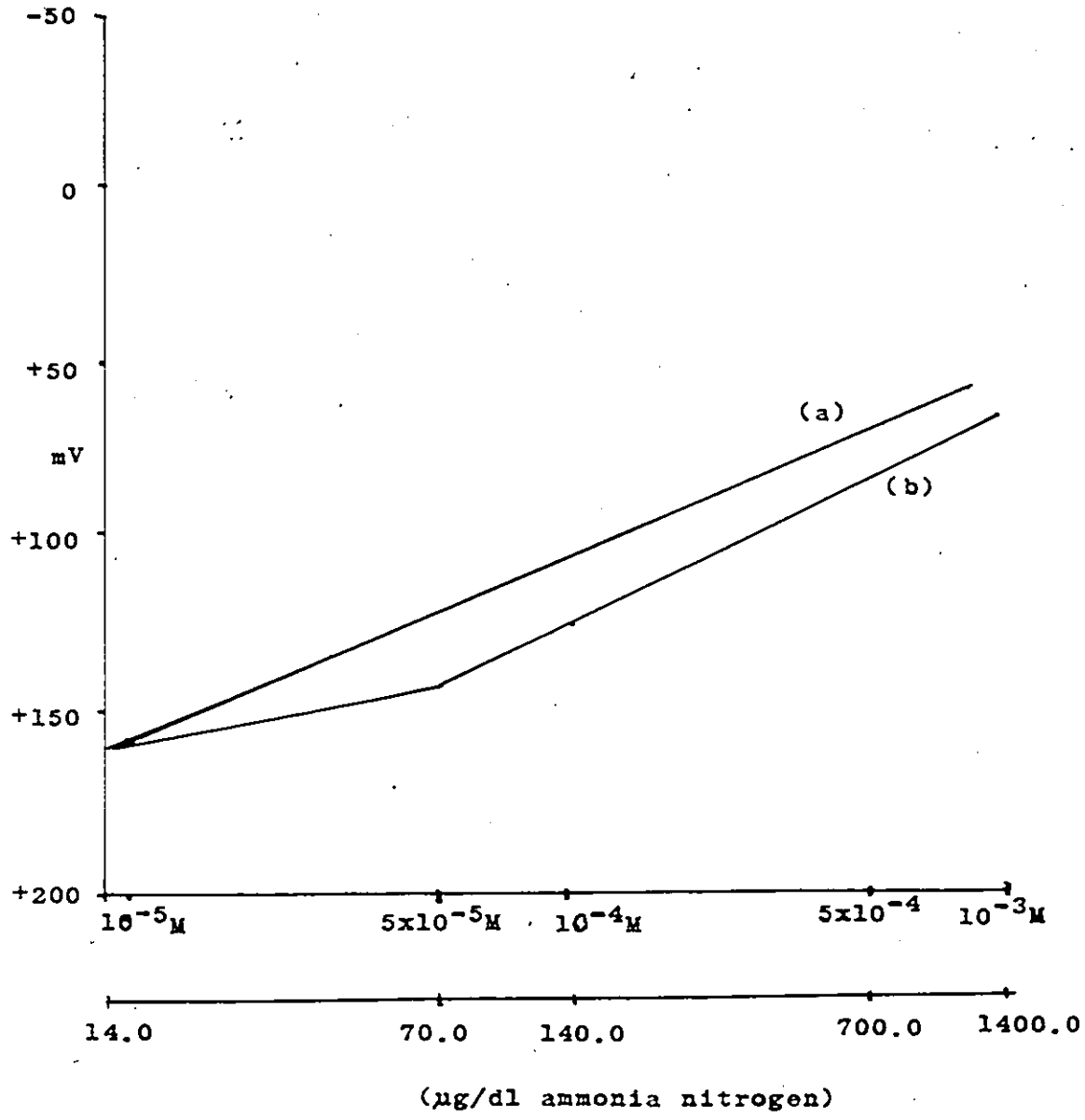
Typical Calibration Curves for the Orion Ammonia-Selective Electrode.

LEGEND

(a) .. Theoretical curve (b) .. Practical curve obtained by Proelss *et al*. (37) and by the automated method in the APPENDIX.

FIGURE 6

Typical Calibration Curves for the Orion Ammonia-Selective Electrode.



addition/subtraction technique. The change in electrode potential observed when a known volume of standard is added to a known volume of unknown is used to calculate the concentration of ammonia in the original sample. A table relating change in potential and concentration is used (Table 4). Also, the observed change in potential can be plotted against the volume of standard added on Gran's plot paper (special semi-antilog graph paper supplied by Orion), and the concentration in the original sample derived from a standard curve. Alternatively, actual concentration can be read directly on a special ion-specific meter. The known addition/subtraction method is the preferred procedure for the analysis of occasional samples, as it does not require the preparation of a calibration curve. However, the level of ammonia in the sample must be known in advance to within a factor of 3 (9).

(b) AUTOMATED METHODS

With the introduction of the second generation "flow-thru" electrodes by Orion, it becomes possible to adapt the Orion ammonia-selective electrode for automated analysis. In 1973 Park and Fenton working in the U. K., reported a simple automated method for estimation of blood ammonia using ISE (38). Figure 7 shows the flow diagram used in that study. The method has a correlation coefficient of 0.979 and a regression equation of $y = 0.939\ x + 5.205$ as compared with ion-

TABLE 4

Values of Q (factor) vs ΔE.

LEGEND

ΔE^a .. change in electrode potential observed.

Q^b .. constant factor.

ΔE^c .. negative changes in electrode potential.

The table is used in the known addition technique and consists of values for a 58mV electrode slope at 25°C.

Taken from Reference 9.

TABLE 4

Values of Q (factor) vs ΔE.

ΔE[a]	Q[b]	ΔE[c]	Q	ΔE[c]	Q	ΔE[c]	Q	ΔE[c]	Q
+2.4	52.6	-3.1	0.415	-8.6	0.186	-18.2	0.0811	-29.2	0.0412
+2.0	5.68	3.5	0.384	9.0	0.178	19.0	0.0767	30.0	0.0394
+1.5	2.66	4.0	0.351	9.5	0.169	20.0	0.0716	31.0	0.0374
+0.5	1.27	5.0	0.297	11.0	0.145	22.0	0.0629	33.0	0.0336
0.0	1.00	5.5	0.276	12.0	0.133	23.0	0.0691	34.0	0.0319
-0.5	0.822	6.0	0.257	13.0	0.121	24.0	0.0556	35.0	0.0304
-1.0	0.696	6.5	0.240	14.0	0.112	25.0	0.0523	40.0	0.0237
-1.5	0.602	7.0	0.225	15.0	0.103	26.0	0.0494	45.0	0.0187
-2.0	0.529	7.5	0.212	16.0	0.0952	27.0	0.0466	50.0	0.0149
-2.5	0.471	8.0	0.191	17.0	0.0884	28.0	0.0440	55.0	0.0120

FIGURE 7

Flow Diagram for Automated Ammonia Analysis According to Park and Fenton.

LEGEND

SMC = single mixing coil.

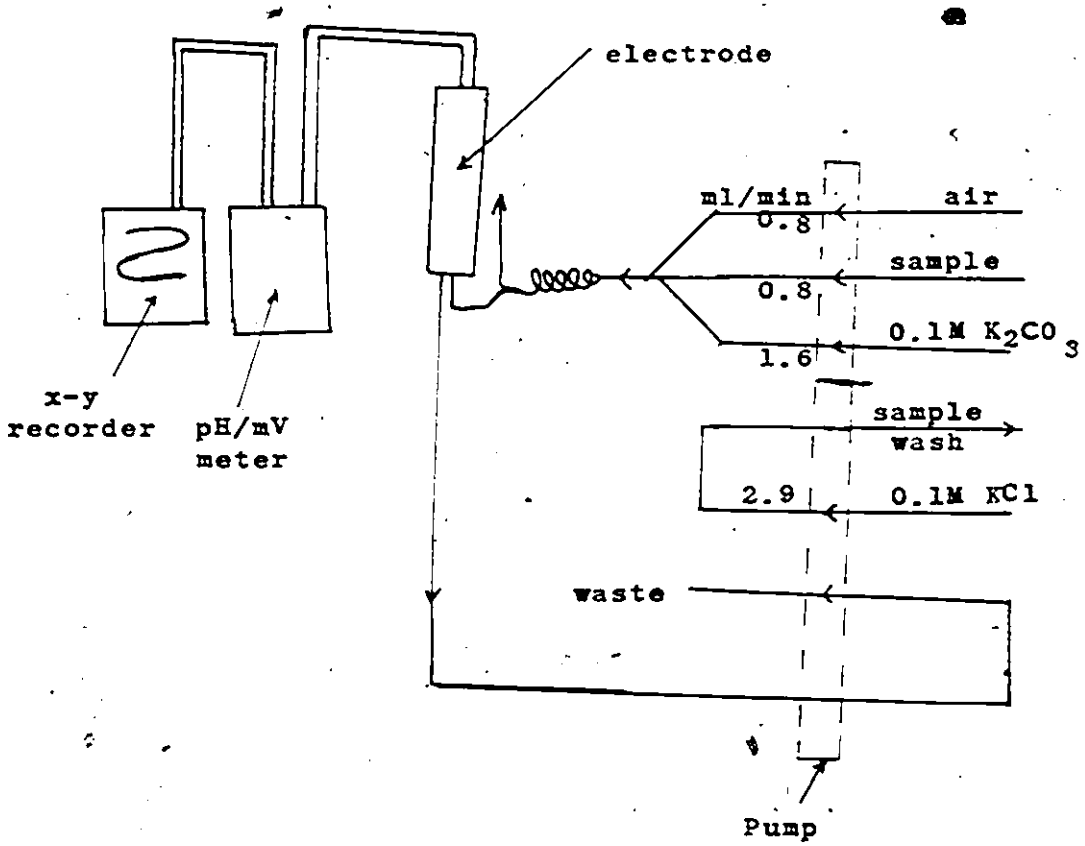
Sampler; 20 per hour.

Cam; 2:1.

Taken from reference 38.

FIGURE 7

Flow Diagram for Automated Ammonia Analysis According to Park and Fenton.



exchange resin method of Fenton and Williams (35). Orion, in recent years, have done a considerable amount of work in this area. Figure 8 (a) and (b) show the flow diagrams currently available from Orion for the automated determination of ammonia in plasma, whole blood and urine (39). Standard equipment required for automated analysis in addition to the electrode is a pH/mV meter with relatively high sensitivity, a 1-10 mV recorder (preferably with a variable chart speed selector), an AutoAnalyzer or similar proportioning pump and a AutoAnalyzer sampler. This equipment although readily available, is relatively expensive and may present a cost problem to smaller laboratories. For larger laboratories where most methods are already automated, existing equipment need only be modified to meet the basic requirements for automated analyses of ammonia in biological fluids.

(4) ADVANTAGES AND DISADVANTAGES OF THE ISE METHODS FOR THE ESTIMATION OF AMMONIA AS COMPARED WITH CONVENTIONAL METHODS

Ion-selective electrode methods are less time consuming than conventional colorimetric methods. The average assay time per sample is 15 minutes and 30 minutes for ISE and resin methods, respectively. The average assay time per sample for the enzymatic method is about the same as for ISE but on a cost per test basis, analyses by the enzymatic technique are more expensive

FIGURE 8

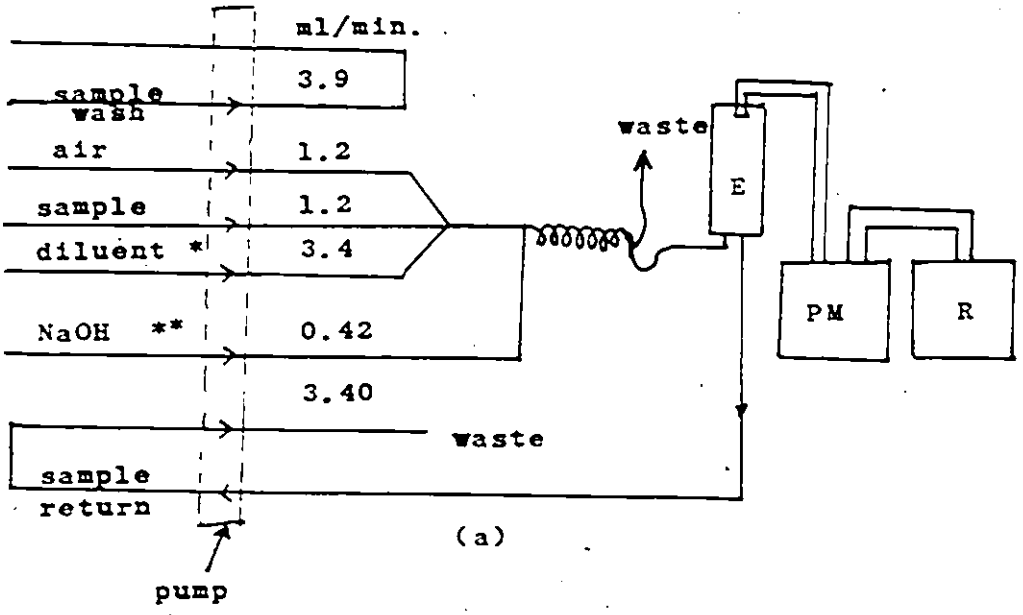
Flow Diagrams for the Automated Ammonia Analysis in Whole Blood or Plasma and Urine.

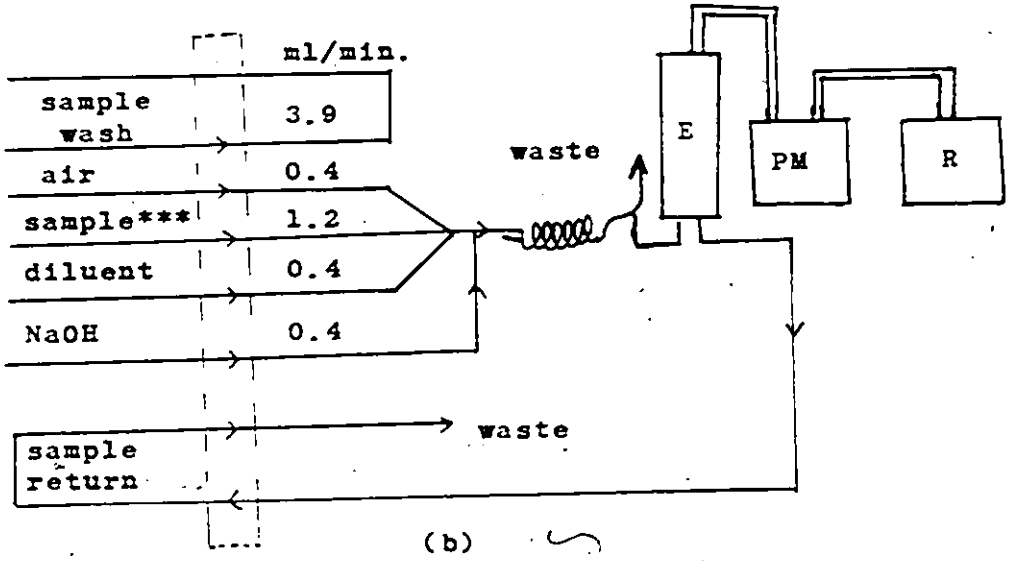
LEGEND

(a) .. whole blood or plasma, (b) .. urine, *.. diluent: 1 x 10^{-5} M NH_4Cl, 0.17 M NaCl, ** .. NaOH: 0.2 M for undiluted plasma, 0.11 M for whole blood diluted 1:2, *** .. Urine sample: diluted 1:250, E = ion-selective electrode, PM = pH/mV meter, R = x-y recorder.

FIGURE 8

Flow Diagrams for the Automated Ammonia Analysis in Whole Blood or Plasma and Urine.





than for ISE. Electrodes are relatively inexpensive and are easily maintained. Since ISE systems respond to ionic activities in solution rather than concentrations, results obtained are closer to the true values of the analytes than for colorimetric or gasometric techniques.

One of the main disadvantages of ISE system for the estimation of plasma ammonia is the interference from labile amino and amide groups from amino acids and proteins. This source of error is not only specific for ISE systems but for all methods used in the estimation of plasma ammonia. However, because of the increased sensitivity and selectivity of the ISE system, the effect is more pronounced. The use of perchloric acid supernate eliminates the effect due to proteins and reduces that of amino acidsto a minimum. The main source of error in the perchloric acid supernate comes from the possible hydrolysis of glutamine. But as pointed out by Proelss and Wright, the hydrolysis of glutamine proceeds very slowly, about 1 µg of ammonia nitrogen per milligram glutamine per hour at 20°C and pH 11 (37). Therefore, the contribution of ammonia from glutamine is considered negligible.

Another possible source of error is the presence of other gases that will diffuse across the membrane and react with water. For example, SO_2 and CO_2 if present in the internal solution, may induce changes in the hydrogen ion concentration, thereby altering the

electrode response. Some organic bases (methylamine and its substituted derivatives) have also been reported to affect the electrode operation. These compounds contain labile amino groups, which in the presence of strong alkali produce ammonia.

(B) DETERMINATION OF SERUM IONIZED CALCIUM

(1) PHYSIOLOGY AND BIOCHEMICAL ROLE OF CALCIUM

Most of the body's calcium is found in bones in conjunction with magnesium as phosphates and carbonates. A small fraction of the calcium is found in blood and other tissues, particularly in muscles. Normally 50% of the blood calcium is bound to proteins, 45% is in the free ionized state and 5% is bound to diffusible ions such as citrate.

It is generally accepted that ionized calcium is the physiologically active form and is responsible for most functions of calcium in the body (40). In additions to the formation of bone, ionized calcium plays special roles in controlling muscle (including the heart) and nerve action, coagulation of blood and cell permeability. The normal level of total calcium in blood ranges between 9-11 mg/dl blood with an average value of around 10 mg/dl. Thus, the normal amount of ionized calcium in the circulation is just below 5 mg/dl. The level of calcium in the circulation is tightly regulated by the action of parathormone, calcitonin, 1,25-$(OH)_2$-Vitamin D_3, the complexing of excess ionized calcium to plasma proteins and citrate and the

excretion of calcium in the urine via the kidneys. Biochemically, ionized calcium functions as an activator in a number of enzymatic reactions. For example, two reactions of universal importance are the calcium dependent hydrolysis of adenosine triphosphate (ATP) and the conversion of prothrombin in the formation of clot:

$$ATP + H_2O \xrightarrow[ATP\text{-}ase]{Ca^{2+}} ADP + H_3PO_4 + ENERGY \quad .... \quad (37)$$

$$Prothrombin \xrightarrow[Thromboplastin]{Ca^{2+}} Thrombin \quad ..... \quad (38)$$

(2) CLINICAL SIGNIFICANCE OF IONIZED CALCIUM

Serum calcium is determined as an aid in the diagnosis of primary hyperparathyroidism, renal impairment and conditions of hypocalcemia in both adults and newborns. The accurate measurement of ionized calcium is also useful in the transfusion of citrated blood. In citrate toxicity, if too much citrate is administered during blood transfusion, the ionized calcium can drop to levels low enough to result in cardiac arrest. Hyperparathyroidism, defined as overly active parathyroid glands, results in elevated levels of parathyroid hormone (PTH) and consequently, hypercalcemia (elevated levels of blood calcium). Calcitonin, another hormone secreted by the thyroid gland, has been demonstrated to lower blood calcium levels. In a large number of cases hyperparathyroidism is associated with normal or slightly increased total calcium and elevated ionized calcium.

In glomerulonephritis, ionized calcium in blood is elevated while that of total calcium is low to normal. In the diagnosis of "Idiopathic Hypercalcuria" (high urine calcium and normal serum total calcium levels) the ionized calcium is usually elevated. The absence of tetany in the presence of low total calcium levels the ionized calcium is usually elevated. The absence of tetany in the presence of low total calcium levels is explained by the increase in ionized calcium due to the presence of severe metabolic acidosis or low serum albumin.

In the newborn, hypocalcemia may occur as a result of drastic metabolic changes at the time of and immediately after birth. Acute changes in acid-base status can lead to rapid changes in the ionized calcium with total calcium remaining normal. Some studies have indicated that when acidosis is corrected with bicarbonate, the ionized calcium is decreased as a function of increase pH. The premature infant is usually a target for multiple biochemical abnormalities such as hypocalcemia, hypomagnesemia, hypoglycemia and anoxia. Thus, the measurement of ionized calcium is considered a better indicator of the patient's status than total calcium. Hypocalcemia in adults may be due to a decrease in plasma proteins resulting from hepatic dysfunction. Thus, ionized calcium determination will serve as an aid in determining whether or not there is true hypocalcemia.

(3) IONIZED OR FREE CALCIUM AS DETERMINED BY ISE

Despite the early recognition that ionized calcium is the physiologically active form, available methods for the direct measurement of this parameter proved to be impractical for routine use. The classical method of McLean and Hastings (40) requires an isolated frog-heart preparation and is therefore not suitable for routine clinical use. Methods in which tetramethyl-murexide is used as a metal-ion indicator, require the separation of proteins and heavy metals before analysis (41 - 43) thus making it difficult to employ these techniques in daily clinical use. Soulier assay (clotting time technique) another method used in the estimation of ionized calcium in serum has never gained wide acceptance in the clinical laboratory. Nomograms for deriving ionized calcium from total calcium and proteins are not always accurate (44). Some Clinicians often favour the corrected calcium instead of nomogram reading:

$$\text{corrected calcium} = \left[\frac{\text{total calcium}}{0.55 + \left(\frac{\text{total protein}}{16}\right)}\right]$$

In 1967 Ross described a liquid ion-exchange ion-selective electrode that can be used for direct measurement of ionized calcium in biological fluids (5). Over the next 6-8 years a variety of experiments, carried out by a large number of investigators, determined the clinical usefulness of the electrode (29, 32). During this period another generation of electrode emerged,

the "flow-through" electrode by Orion. This electrode has several advantages over the static type electrodes. These include more rapid equilibrium, increased stability, less daily drifts, smaller volume of sample and the anaerobic measurements of sample similar to blood pH measurement. We have successfully used the Orion flow-through type calcium electrode (series 99-20) to measure ionized calcium in several normal and renal dialysis patients.

(4) ANALYTICAL PROCEDURE

Serum is recommended since common anticoagulants such as heparin and oxalate complex calcium as well as inhibiting blood clotting.

The procedure for the flow-through type electrode is relatively standard. Venous blood is collected, centrifuged and the serum is transfered anaerobically to a syringe (a 1.0-ml tuberculin syringe is ideal). The syringe is then attached to the Luer lock of a pump which is connected to the calcium-sensitive and reference electrodes. The sample is then pumped slowly through the electrode and the potential change is observed on a mV/pH meter. The assembled electrode is shown diagramatically in Figure 9. Standards and sample are treated alike and the concentration of the unknown is read from the standard curve prepared from millivolts versus concentration on semi-log graph paper. Landenson et al. and Burr utilized this technique in studies with the Orion flow-through ionized

FIGURE 9

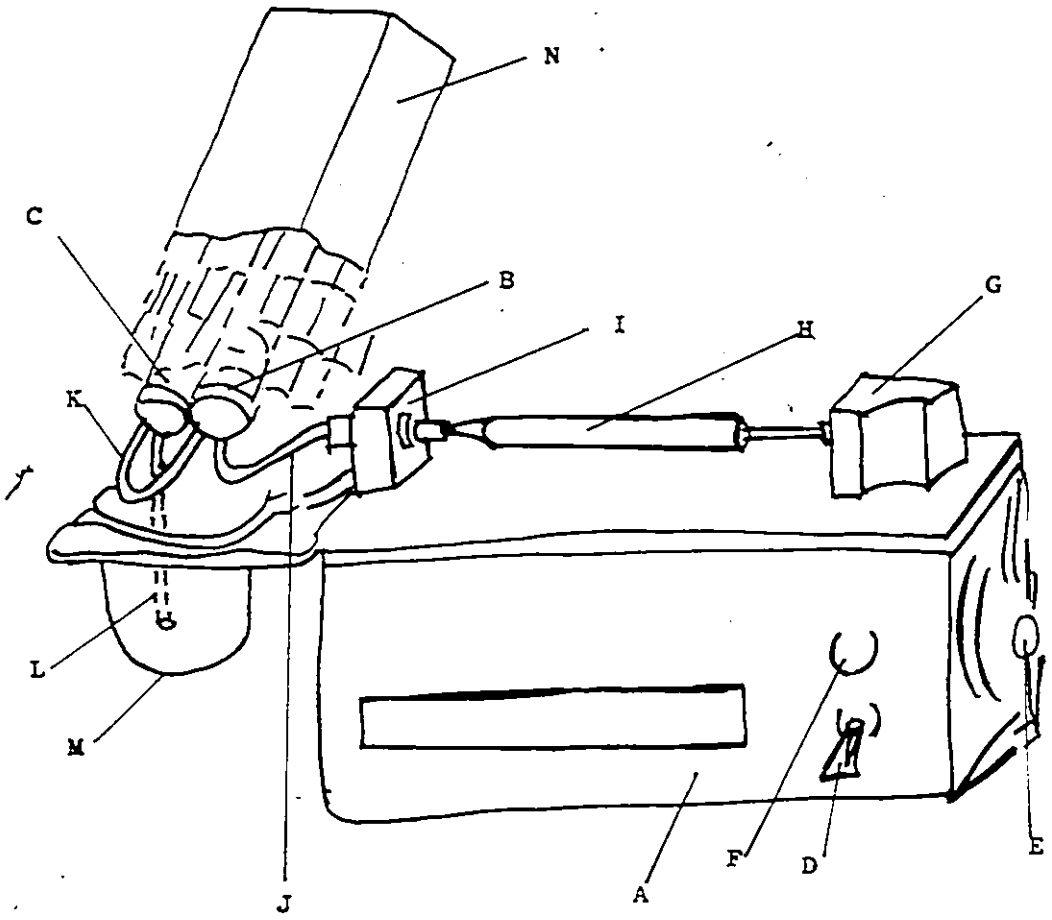
Diagramatic Representation of the Orion Calcium Ionalyzer ®.

LEGEND

This diagram is taken from reference (20) without permission of author. A .. Syringe pump; B.. Calcium electrode; C .. Reference electrode; D .. Pump motor ON/OFF switch; E .. Drive wheel; F .. Pilot light; G .. Syringe drive block; J, K, L plastic tubing - transports sample between syringe and calcium electrode (J) and between calcium electrode and reference electrode (K). Larger plastic tubing - transports sample from reference electrode to beaker (L); M .. Plastic waste beaker; N .. Electrostatic shield.

FIGURE 9

Diagramatic Representation of the Orion Calcium Ionalyzer ®.



calcium-selective electrode (45, 46).

Orion recommends the addition of three drops of triethanolamine solution (1 M) and 0.06 g of trypsin to 100 ml of standard solution to produce the desired pH of 8. Several workers have found that an error is introduced by the use of triethanolamine (46, 47). Thus, there is an uncertainty existing with respect to standardization of the calcium electrode for measurement in biological specimens. The effect of this base on calibration of the electrode was not studied in our laboratory. We found the calibration curve to be satisfactory when using triethanolamine and trypsin as recommended by the manufacturer.

Another procedure described by Schwartz as a new technology and methodology in ionized calcium electrodes, is the automated solid-state, calcium-specific dip electrodes (47). (This system is available commercially from Applied Medical Technology, Box 689, Menlo Park, California. 94025.) The system is capable of permitting measurements on thirty samples with up to six different electrodes simultaneously. It represents a new approach in electrode measurements and its success in the daily clinical use is yet to be evaluated.

More recently Orion introduced the Space Stat 20 ionized calcium analyzer (48). This instrument is now being used routinely for the analyses of ionized calcium

in several laboratories. Only one calibration per day is necessary. The instrument standardizes, measures and washes out automatically. Results are displayed as digital output in meq/L three minutes after pressing the start button. Measurements are made on 500 µl of whole blood and the Space Stat 20 is said to have a precision of ± 0.01 meq/L.

(C) DETERMINATION OF SODIUM AND POTASSIUM IN BLOOD AND URINE

Serum sodium and potassium are used in conjunction with other electrolytes as an aid in the diagnosis of electrolyte and water imbalances, e.g. in dehydration, edema, Addison's disease and renal malfunctions. Determination of sodium and potassium is also useful in diagnosing hypertension associated with hypokalemia.

(1) ANALYTICAL PROCEDURES

(a) MANUAL METHODS

The use of ion-selective electrodes for the determination of sodium and potassium dates back to the initial work on solid-state glass membrane electrodes by Eisenman et al. (2). Manual methods are considered impractical for routine clinical use. One of the main problems with glass electrodes is their relatively broad sensitivity to certain cations. However, with glass prepared specially for sodium ion detection, the use of these sensors in clinical usage becomes more apparent. Because of the high level of sodium to potassium in

serum (about 25:1), the sodium ion electrode need only be selective for sodium without suffering any interference from potassium. As for the determination of potassium in the presence of sodium, the situation is less straight-forward. A much higher selectivity ratio of potassium over sodium (at least 1000:1) is required. Early glass electrodes did not even come close to this figure. However, it has been known for sometime that certain macrocyclic antibiotics are excellent complexing agent for potassium. Working on this observation, Stefanac and Simon et al. (6) invented a new type of electrode using Valinomycin as membrane material. A selectivity ratio of better than 38,000:1 for potassium over sodium has been reported for this electrode (6).

(b) AUTOMATED METHODS

With the introduction of the "flow-through" type electrode, sodium-ion glass electrodes and the potassium ion-selective Valinomycin electrodes are now employed in automated systems and used routinely in several laboratories. For example, the Technicon Stat/Ion system, a multi-channel automated electrolyte analyzer, is already being used in several laboratories across Canada. It is a fully automated system which makes use of ion-selective electrodes to determine sodium, potassium and chloride in both serum and urine. Precision and accuracy studies reported by the manufacturer are shown in Table 5 (40). These values are exceptionally

TABLE 5

Reproducibility and Precision Data for the Technicon Stat/Ion Electrolyte Analyzer.

LEGEND

The regression analyses for sodium and potassium are derived from comparison studies between the Stat/Ion Analyzer vs the AutoAnalyzer II. Regression analysis for chloride is derived from comparison between the Stat/Ion Analyzer and the manual thiocyanate method. *Regression analyses and correlation coefficient have not been stated for urine samples. r = correlation coefficient.

TABLE 5

Reproducibility and Precision Data for the Technicon Stat/Ion Electrolyte Analyzer.

Sample	Analyte	Mean meq/L	SD	%CV	Regression Line	r
SERUM	Sodium	165	0.578	0.35	y = 0.972x + 0.09	0.997
		120	0.489	0.41		
	Potassium	9.6	0.122	1.44	y = 0.972x + 0.09	0.997
		2.0	0.053	2.65		
	Chloride	135	1.038	0.77	y = 1.002x + 0.9	0.981
		80	0.612	0.77		
URINE	Sodium	163	0.28	0.17	*	*
	Potassium	56.1	0.12	0.22	*	*
	Chloride	181.8	3.0	1.6	*	*

good in comparison with conventional methods. Similar results were reported for urine analyses. Another multi-channel automated system which has been in clinical use for three years and is very similar to the Technicon system is the Photovolt Electrolyte Analyzer PVA-4. Like the Technicon system, the PVA-4 is capable of doing 48 samples (192 tests) per hour and only requires 0.3 ml of serum. Precision reported for the PVA-4 is as follows:

Na^+, Cl^-, HCO_3^- $\pm$ 1mM/L

K^+ $\pm$ 0.1mM/L

Linearity ranges are 20-299 mM/L for Na^+ and Cl^-, 2-12/20-199 mM/L for K^+ (for serum and urine, respectively) and 8-40 mM/L for bicarbonate. These are similar to those stated for the Technicon system.

Similar electrode systems are used in the Technicon Simultaneous Multiple Analyzer with Computer (SMAC) for determination of sodium and potassium which is coming into more widespread use in a number of countries.

(c) SEMI-AUTOMATED METHODS

Based on the calcium Space Stat 20 Calcium Analyzer, Orion has manufactured and marketed a semi-automated analyzer for sodium and potassium. This system functions analogous to the calcium analyzer and measures sodium and potassium in biological samples by means of ion-selective electrodes. Unlike the conventional flame photometer, the Orion SS/30 requires no centrifugation of the sample prior to analysis. Whole

blood is used directly. The instrument is reported as being very reliable, requires little maintenance and is ideal for stat work.

(D) DETERMINATION OF BLOOD UREA NITROGEN (BUN)

Clinically, the estimation of blood urea nitrogen is used as an aid in differential diagnosis of liver and kidney diseases. The normal value of BUN is about 10-20 mg/dl. Levels of 50-150 mg/dl are indicative of severely impared renal function, while levels greater than 150 mg/dl are conclusive of serious glomerular dysfunction. A low BUN is frequently associated with states of over-hydration (50).

Current methods of analysis of BUN in routine clinical uses are those in which BUN reacts with diacetyl monoxime in acid solution to form a complex, which in the presence of a variety of reducing agents yield a coloured product.

More recently, an enzyme-electrode system has been developed for the determination of blood urea nitrogen (8). Urease is immobilized on an alumina cartridge which in turn forms part of an ammonia-selective electrode system. The sample is injected through the cartridge where BUN is converted to ammonia. (This system is available commercially by Kimble, a division of Owens, Illinois, U.S.A.).

The ammonia produced is measured directly by the electrode as discussed in section (A), (3). The

principle of operation is the same as that used in the urea transducer of Guilbault et al. (13). The Kimble BUN analyzer is said to possess a precision of $\pm$ 1 mg/dl and is capable of doing 30-45 tests per hour.

(E) DETERMINATION OF CHLORIDE IN BIOLOGICAL SPECIMENS

In addition to serum, chloride is usually determined in spinal and other body fluids, in urine and in sweat. Sweat chloride is of special clinical interest as an aid in the diagnosis of cystic fibrosis, a rare inherited disorder in neonates. Gravimetric methods for determining chloriae in sweat are tedious and frequently inaccurate. With the introduction of the chloride ion-selective electrode, direct measurement of sweat chloride is possible. A portable specific-ion meter combination is marketed by Orion and is currently used in our laboratory. We have found this system to perform satisfactorilly. It is easy to operate and does not require a skilled technologist. The electrode is a solid-state combination type discussed in CHAPTER 11 and possess a silver silver-chloride membrane.

As previously mentioned, ion-selective electrodes are employed in the automated systems of Technicon and Photovolt for the determination of chloride in serum and urine. These sensors, like the Orion chloride-selective electrodes, possess membranes of silver chloride specifically designed to sense chloride ions in solution.

(F) DETERMINATION OF BLOOD GLUCOSE LEVEL

Potentiometric determinations of blood glucose

date back to the early methods of Clark utilizing the oxygen electrode. The electrode was designed to measure oxygen, but can be adapted into ISE systems which measure glucose utilization. This system is successfully employed in the Beckman blood glucose analyzer currently in use in many laboratories. More recently, glucose oxidase and peroxidases are immobilized on special matrix materials. The reaction mixture is then allowed to flow through the matrix with subsequent catalysis by the immobilized enzyme. The products formed are monitored by a variety of techniques. One possible technique suggested is the incorporation of a pH electrode in the system to measure the change in hydrogen ion concentration as glucose is converted into gluconic acid. The pH electrode has been successfully used in other automated systems. (51). In addition to increased specificity, the immobilized enzymes are stable for several months and can be reused for several hundred highly accurate and reproducible assays (52).

The immobilization of enzymes and related materials on suitable inert matrices as part of an electrode or non-electrode system, represents a relatively new concept in analytical chemistry. Its general applicability in routine clinical use is yet to be established.

CHAPTER IV

DISCUSSION AND CONCLUSION

(A) POTENTIAL SOURCE OF ERROR OF INDICATOR AND REFERENCE ELECTRODES

The common source of error with respect to the indicator electrodes have been described in previous chapters. To recapitulate, ISE are sensitive only to free ions in solutions. Thus, if ions are in insoluble or complexed forms, ISE systems will produce erroneous results. The presence of proteins or other organic compounds may effect the electrode response by their interactions with the membrane. Another common source of error is that caused by the presence of undesired ions. Selectivity ratios are often supplied by various manufacturers of ISE, but these ratios are specific for the ions that are known to interfere with particular electrodes. One cannot obviate the possibility of interferences by diverse cations. In continuous analyses, one assumes rapid equilibrium processes on both sides of the membrane. Thus, automated techniques are most suited for those constituents in which movement across the membrane is relatively fast.

Reference electrodes used in ion-selective electrodes for clinical purposes are the silver/silver-chloride and the mercury/mercurous chloride (calomel) electrodes. One source of error common to all reference electrodes is that

pertaining to the junction between the internal reference solution and the sample solution. Variation in residual liquid junction potentials is a complex problem and will not be discussed further in this study.

(B) OTHER AREAS IN THE CLINICAL CHEMISTRY LABORATORY IN WHICH ION-SELECTIVE ELECTRODES MAY BE USEFUL

Ion-selective electrodes capable of measuring lead, copper and cadmium in solution have been discussed by Covington and others (10). Most laboratories at present are determining heavy metals in serum and urine by atomic absorption. Although atomic absorption methods are usually precise and accurate, they are often quite tedious and require relatively expensive equipment. Atomic absorption techniques also suffer from a matrix effect and in many analyses, filtrates are required. ISE techniques offer a good alternative to atomic absorption. However, the full potency of ISE as methods of determining heavy metals (lead, mercury, cadmium, zinc, iron and copper) in biological fluids may not be realized until present electrode systems are modified.

ISE systems currently employed in industrial, health and biochemical research, such as fluoride and bromide electrodes, can be easily adapted for routine clinical use. Fluoride is not usually assayed routinely in laboratory specimens while bromide in serum is only requested by physicians occasionally. Thus, the

application of these sensors in clinical laboratories is of no apparent significance at present.

Magnesium, manganese and cyanide are other important ions which may be accurately determined in biological specimens by ISE.

(C) ACCURACY, PRECISION AND GENERAL APPLICABILITY OF ISE

It is very difficult to assess absolute accuracy for ISE methods since there is no independent method for direct corroboration. However, one may assume relative accuracy if the variability and reproducibility between duplicates are low, the system is highly selective to the ion of interest, interference from other ions is negligable and percent recoveries are good.

Precision, a direct measurement of the reproducibility of the system, is more meaningful than relative accuracy for all practical purposes. Usually most electrode systems possess precisions (expressed as percent coefficient of variation) of better than 2%. Precision is calculated from a number of determinations performed on a given sample in duplicate or triplicate as follows:

$$\text{Standard Deviation (SD)} = \sqrt{\frac{\sum(X-\bar{X})^2}{N}} \quad \ldots\ldots\ldots \quad (39)$$

$$\%\ \text{Coefficient of Variation (\%CV)} = \left(\frac{SD}{\bar{X}} \times 100\right) \ldots \quad (40)$$

X is the measured value of the parameter, $\bar{X}$ is the mean and N is the number of duplicates.

The widespread application of ISE in the clinical

laboratory is now apparent. The technique is no longer used to measure parameters that are difficult to measure by conventional methods. This is particularly true in the case of BUN and electrolytes for which very reliable colorimetric, electrometric, and photometric methods exist.

(D) CONCLUSION

I have presented in this paper a general overview of the various types of ISE and their application in the clinical laboratory. Although it is shown that these electrodes can be successfully used in routine analyses, there are still some unsolved problems. For example, there is an uncertainty as to the effect of pretreating calcium standards before estimation of ionized calcium with the liquid ion-exchange electrode. Some authors favour pretreatment with ethanolamine and trypsin while others do not. Studies have indicated that a progressive decrease in ionized calcium concentration occurs when trypsinized standards have been stored for longer than six hours at room temperature (53). Users of ISE systems have come to realize the full usefulness of these sensors over corresponding colorimetric techniques. The fact that they have to accept near Nernstian responses for most practical electrodes is of no great significance. Usually the high sensitivity, selectivity and precision obtained with ISE systems make them worthwhile.

The present and future success of the ISE in routine clinical use, however, lies in their ability to function in automated systems, in both single and multiple channel instruments. Already the number of such units in the clinical chemistry laboratory has increased significantly over the last few years.

APPENDIX

THE AUTHOR'S EXPERIENCE WITH TWO ION-SELECTIVE ELECTRODES IN A "MID-SIZE" CLINICAL CHEMISTRY LABORATORY

My interest in ISE began in eary 1973. Shortly after joining the staff at Grace Hospital, Windsor, I started evaluation studies on the Orion Calcium Ionalyzer®. Our main intent has been to use this system to determine ionized calcium in cases of abnormally high or low total calcium. This then lead to further studies on the applicability of the Orion ammonia-selective electrodes (static and flow'through') systems. The following is a summary of my experience with these two systems.

(A) AMMONIA-SELECTIVE ELECTRODE

All attempts to use the electrode as a static dip type electrode according to the manufacturer operating procedure (9) have failed. Reproducibility has been poor, leakage of the internal filling solution has caused the electrode readings to drift and the slope of the standard curve has been considerably less than the theoretical value. Although I have been able to obtain results by the known addition procedure as opposed to the direct measurement by standard curve, these are often inaccurate and unreliable. Similar problems have been encountered when I try the method of Proelss and Wright (37). With the introduction of a "flow-through" cap for this electrode, I have decided to try automation. The method

of Proelss and Wright has been adapted for this study (37).

(1) MATERIALS AND METHODS

(a) MATERIALS

(i) REAGENTS: All reagents, unless otherwise stated were reagent grade.

(ii) SOLUTIONS: All solutions prepared in ammonia-free distilled water.

(iii) AMMONIA-FREE WATER: Prepared as for the ion-exchange resin method (54).

Internal filling solution, diluent, stock and working standards (NH_4Cl), perchloric acid, sodium hydroxide and electrode storage solution were prepared according to Proelss and Wright (37) except that working standards were prepared in 8% perchloric acid.

(iv) INSTRUMENTS

Technicon AutoAnalyzer AA1 sampler and pump; ammonia electrode, "flow-through" type (model 95-10, Orion Research). Radiometer pH/mV meter (model pH meter 26). John's Scientific Recorder (model M521). Technicon AutoAnalyzer tubings, sample probe and single mixing coils.

(v) SPECIMEN COLLECTION AND PRESERVATION

All specimens were collected in heparinized vacutainers, kept on ice and taken to the laboratory as soon as possible. Proteins were precipitated with chilled perchloric acid, the sample centrifuged at 2000 - 3000xg for 5 minutes and the protein-free filtrate kept

on ice until assayed.

(b) METHOD

The electrode is assembled as previously reported (39) and the AutoAnalyzer set up as shown in Figure 10. The pH/mV meter is set to pH mode and the diluent is then run through the system until a stable base line is obtained. The recorder is set to 10 mV for full scale deflection and chart speed of 0.25 cm/min. Standards and unknowns (perchloric acid supernates, 1:2 dilution) are then assayed and the concentration of the unknown is read from a standard curve.

(2) STANDARDIZATION

Electrode readings for a series of standards is plotted as peak height vs. concentration on semi-log graph paper. Figures 11 and 12 show an AutoAnalyzer tracing and a standard curve, respectively, obtained for the ammonia electrode.

(3) COMPARISON STUDIES

Correlation studies between the ISE method and an ion-exchange resin method (54), and an enzymatic method (55) were performed. The regression lines for comparative data (Table 6 and 7) between ISE vs. ion-exchange resin and ISE vs. the enzymatic method are $y = 0.688x + 9.830$ and $y = 0.340x + 22.260$, respectively. The correlation coefficients (r) for the two sets of data are 0.947 and 0.495, respectively. The correlation between ISE and resin method is in close agreement with

FIGURE 10.

Flow Diagram Used in The Clinical Chemistry Laboratory at Grace Hospital for Whole Blood or Plasma Ammonia Determination.

LEGEND

* .. Sample; perchloric acid supernate (1-2 dilution)

** .. Diluent; 0.25 M Na_2SO_4, 0.17 M NaCl, 10^{-5} M NH_4Cl; *** .. NaOH; 11 M NaOH; E = ISE; PM = pH meter; $\dot{w}$ = waste; Cam: 50-2:1, SMC = single mixing coil.

FIGURE 10

Flow Diagram Used in The Clinical Chemistry Laboratory at Grace Hospital for Whole Blood or Plasma Ammonia Determination.

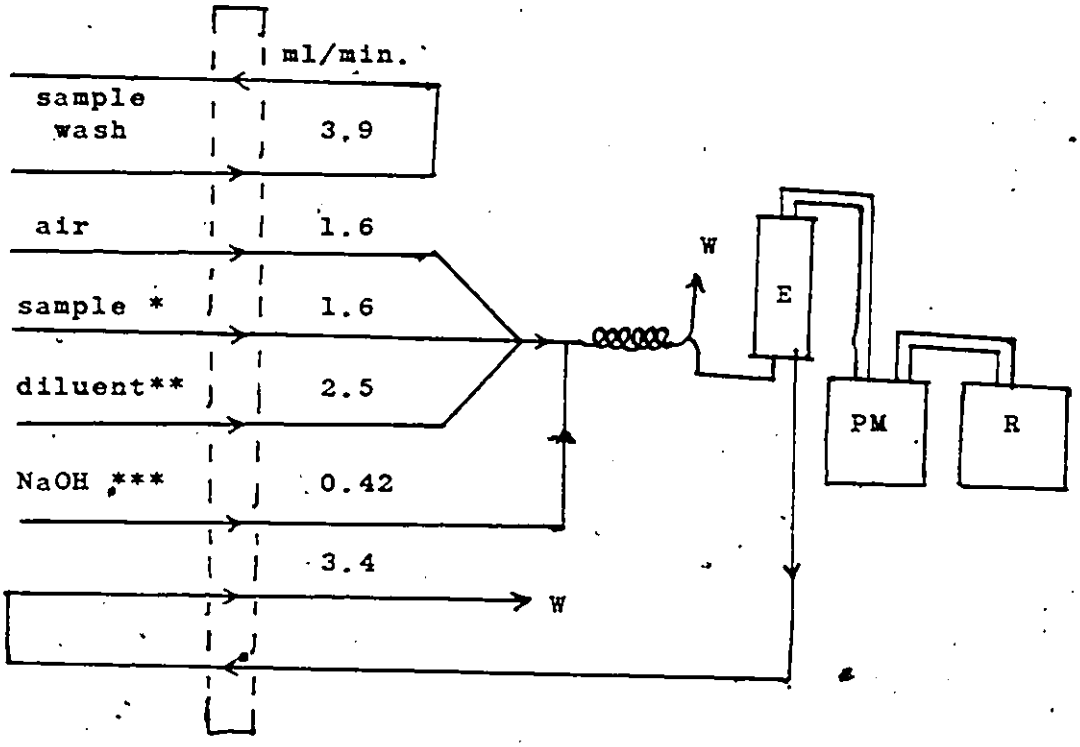


FIGURE 11

AutoAnalyzer Tracing for Ammonia Standards.

LEGEND

Working standards were prepared from a 10^{-1} M NH_4Cl stock by serial dilution in 8% perchloric acid to obtain the following: 1.0×10^{-5} M, 0.5×10^{-4} M, 1.0×10^{-4} M, 0.5×10^{-3} M, 1.0×10^{-3} M, 0.5×10^{-2} M.

FIGURE 11

AutoAnalyzer Tracing for Ammonia Standards.

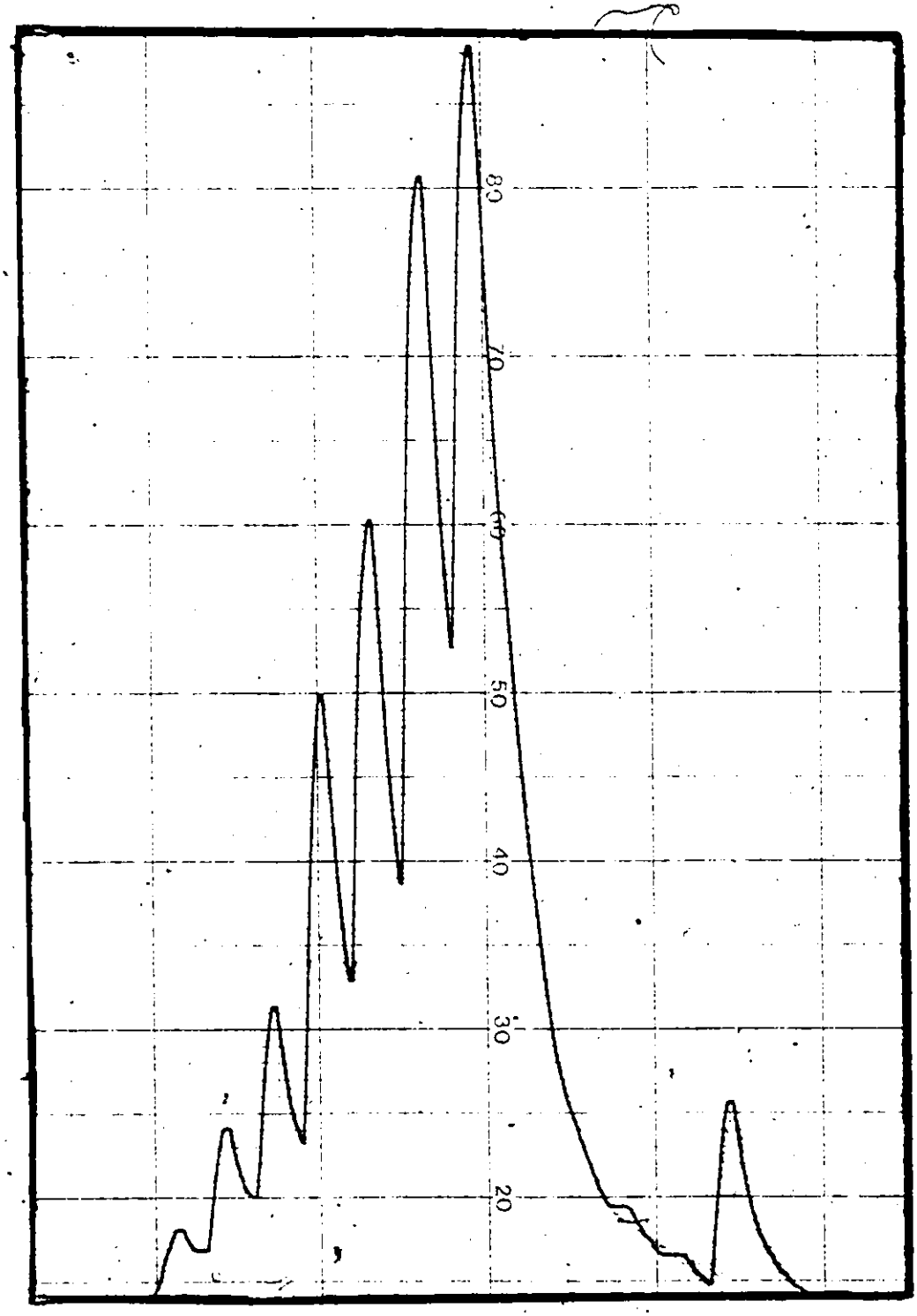


FIGURE 12.

Calibration Curve for the Automated Analysis of Ammonia.

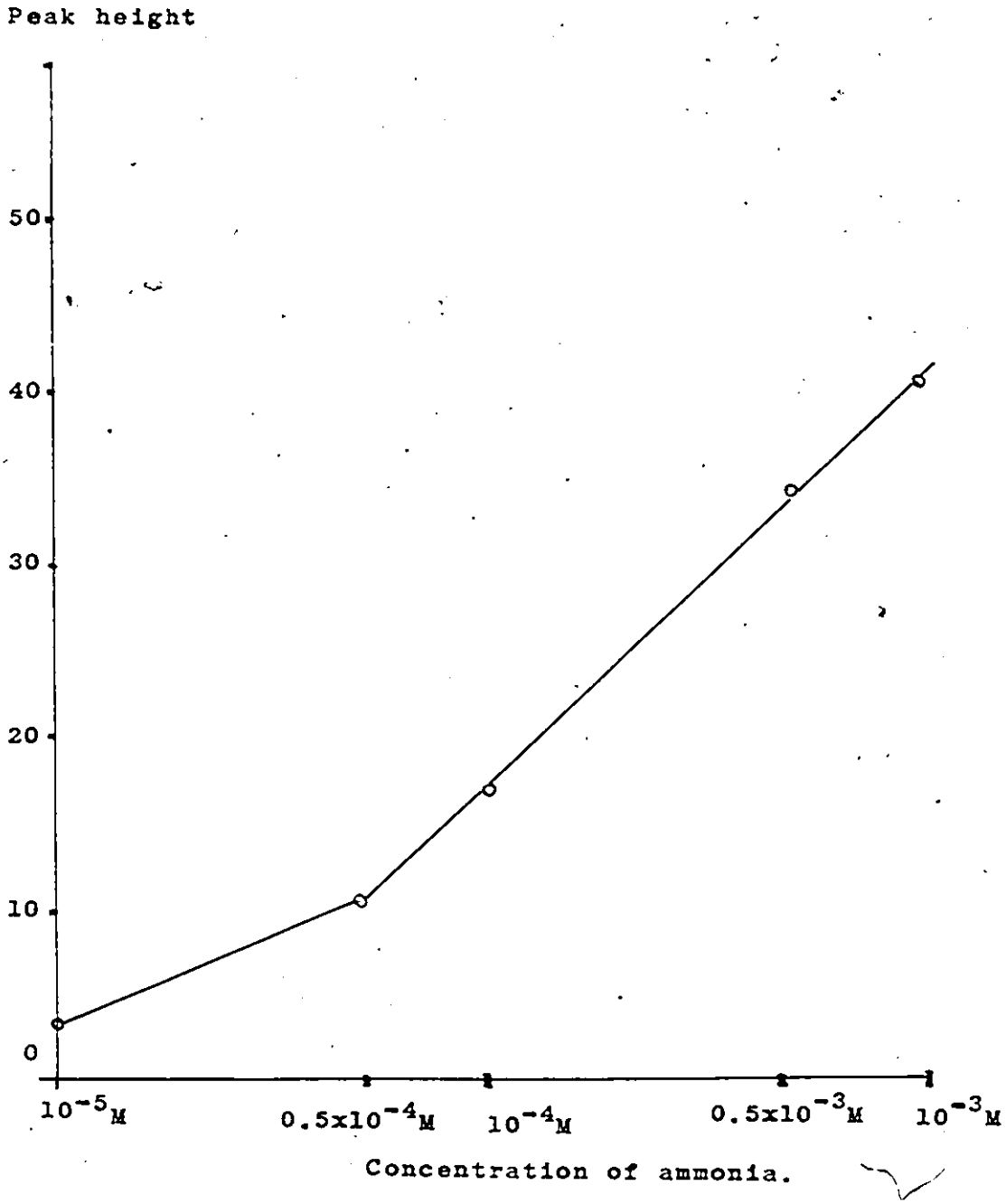


TABLE 6

Comparison Studies Between the ISE and Eskalab Enzymatic Method for Ammonia Determination.

LEGEND

$y = 0.340x + 22.26$ (regression equation)

$r = 0.495$ (correlation coefficient)

The results shown in Table 6 represent means of duplicate assays carried out on samples from 10 normally healthy individuals by the enzymatic method (55) and the automated ISE method.

TABLE 6

Comparison Studies Between the ISE and Eskalab Enzymatic Method for Ammonia Determination.

Patient #	µg/dl Ammonia N (Enzymatic)	µg/dl Ammonia N (ISE)
1	46	51
2	55	48
3	49	37
4	27	31
5	-	31
6	13	24
7	29	26
8	21	41
9	34	26
10	51	27

TABLE 7

Comparison Between the ISE and Ion-Exchange Resin Method for Ammonia Analysis.

LEGEND

$y = 0.688x + 9.83$ (regression equation).

$r = 0.947$ (correlation coefficient).

Samples from 10 normally healthy individuals (male and female in the age range of 15 - 75) were assayed by the ion-exchange resin method (54) and the automated ISE method. The averages of duplicate assays are shown in Table 7.

TABLE 7

Comparison Between the ISE and Ion-Exchange Resin Method for Ammonia Analysis.

Patient #	µg/dl Ammonia N (Resin)	µg/dl Ammonia N (ISE)
1	43	37
2	40	37
3	34	35
4	25	25
5	28	25
6	18	24
7	32	32
8	20	25
9	41	41
10	44	41

those obtained by Proelss and Wright (37). It is difficult to explain the relatively low correlation between ISE and the enzymatic method. The enzymatic method is known to produce higher results than the ion-exchange resin. This appears to be true for ISE also, as can be seen from the regression equation.

(4) PRECISION

Within and between-run precision as determined on a 140 µg/dl ammonia nitrogen standard are well within the acceptable limits for ammonia analysis. A normal range of 14-58 µg/dl (mean $\pm$ 2SD) was obtained for this automated method.

(B) CALCIUM IONALYZER®

(1) MATERIALS AND METHODS

All materials were prepared as stated in the instruction manual (20). Stock standards ($CaCl_2$) were purchased from Orion. Working standards were prepared according to the instruction manual.

(a) INSTRUMENTS

A Radiometer pH/mV meter (model pH meter 26) and Orion Ionalyzer® (model 99-20) were used in this study.

(b) METHODS

The system was assembled as shown in Figure 9 and the analyses performed as per manufacturer's manual.

(2) STANDARDIZATION

Figure 13 shows the theoretical calibration curve for the calcium ion-selective electrode. Figure 14 is

FIGURE 13

Typical Calibration Curve for the Orion Calcium Ionalyzer®.

LEGEND

This calibration curve was redrawn from reference 20 without the author's permission.

FIGURE 13

Typical Calibration Curve for the Orion Calcium Ionalyzer®.

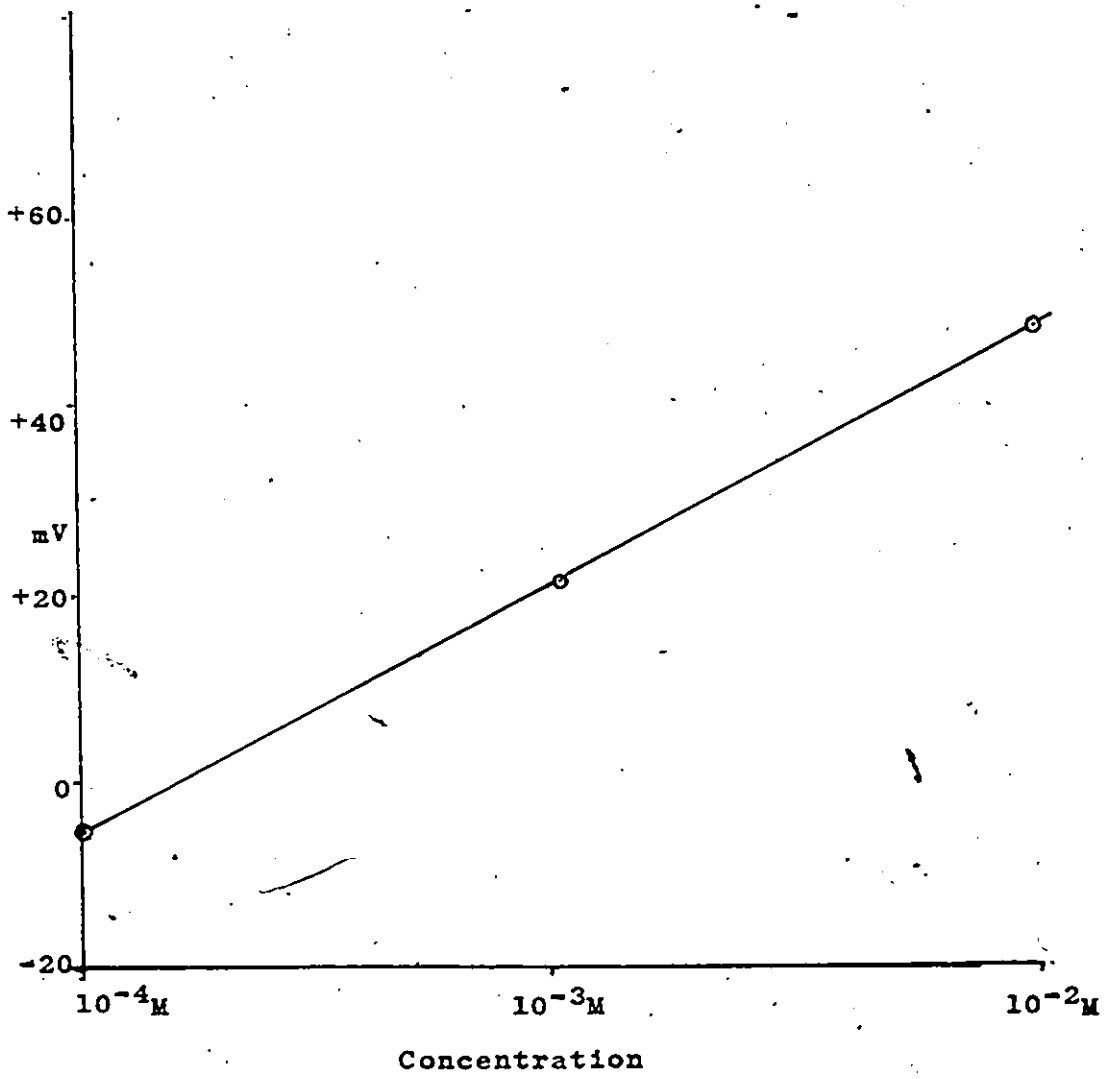


FIGURE 14

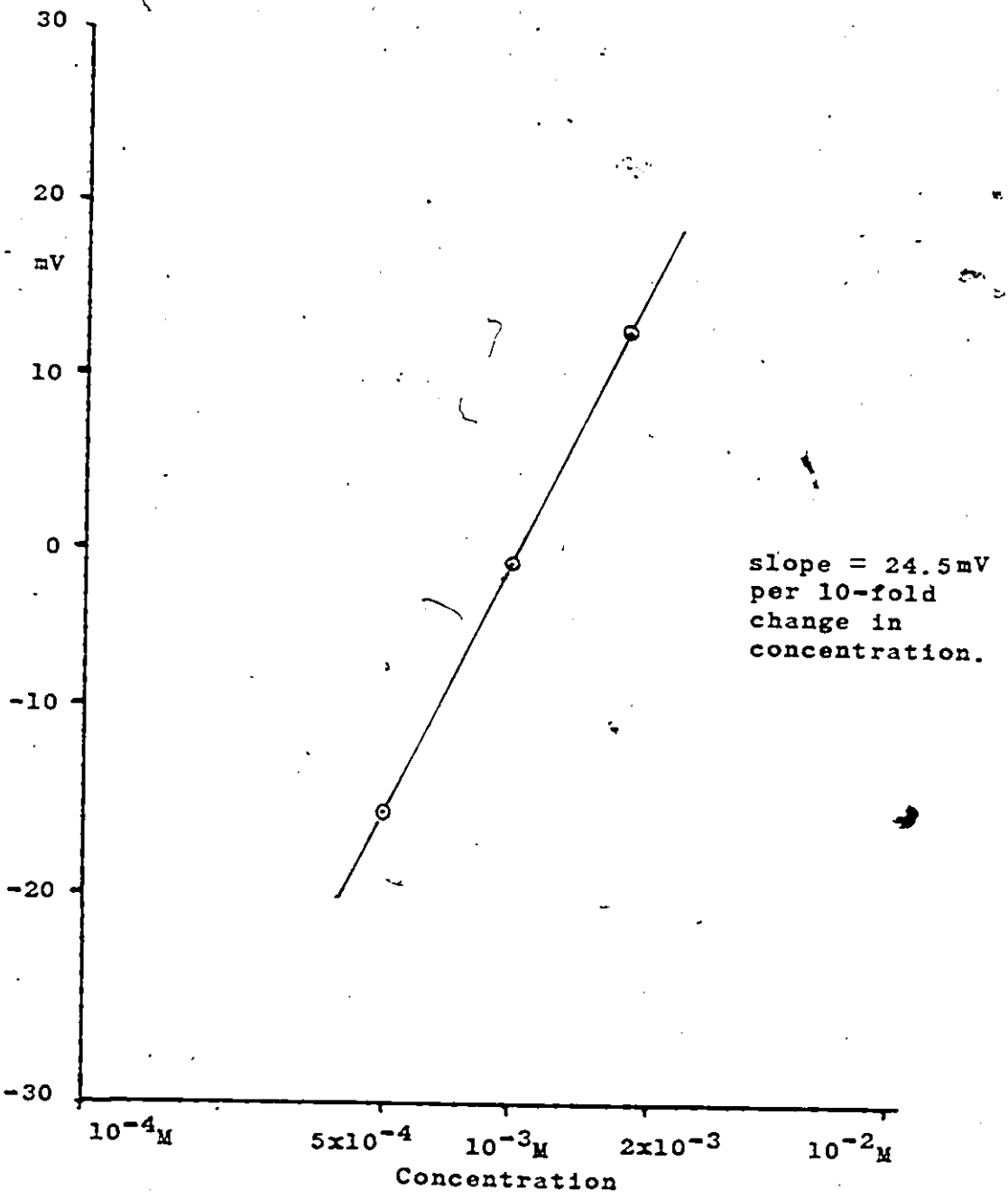
Standard Curve Obtained in The Clinical Chemistry Laboratory at Grace Hospital for the Calcium Ionalyzer®.

LEGEND

A slope of 24.5 mV per 10-fold change in concentration at room temperature was obtained for the ISE system. Trypsin and ethanolamine were added to the working standards to give desired pH of 8, as recommended by the manufacturer.

FIGURE 14

Standard Curve Obtained in The Clinical Chemistry Laboratory at Grace Hospital for the Calcium Ionalyzer®.



the standard curve obtained in our laboratory. The slope of this line is 24.5 mV at room temperature. Theoretically, a slope of 29.5 mV at 25°C is expected. Thus, the electrode response was considered satisfactory.

(3) COMPARISON STUDIES

Ionized calcium data by ISE (Y) and the nomogram (X) method for 16 patients with low, normal and elevated total calcium (see Table 8) were compared. The regression line obtained is $y = 0.425x + 1.273$ and a correlation coefficient (r) of 0.381, which indicated a lack of correlation between the two methods. As previously mentioned, nomograms often produce inaccurate results and are therefore unsuitable for use as reference methods. This finding agrees with the findings of others.

(4) PRECISION

Repeated assays on control and patient sera have been used to determine the reproducibility of the calcium Ionalyzer®. Excellent within-run ($\pm$ 2%) and between-run ($\pm$ 2.6%) precision were obtained.

(C) COMMENTS

(1) RECOVERY STUDIES

Because of the failure of the ammonia electrode to function as a single sample electrode, no recovery studies have been performed by this technique. However, recovery experiments have been set up for the automated version. The average percent recovery obtained in our laboratory is 102%. Unfortunately,

TABLE 8

Comparison Data Between the Nomogram and ISE Methods for Determining Ionized Calcium.

LEGEND

* Total calcium was determined by the Technicon AA1 complexone method.

$y = 0.425x + 1.273$ (regression equation).

$r = 0.381$ (correlation coefficient).

Samples from 17 patients with elevated, normal and lowered serum total calcium were assayed (see method) in duplicate by the nomogram method of McLean *et al.* (54) and by the Orion Ionalyzer® (20).

TABLE 8

Comparison Data Between the Nomogram and ISE Methods for Determining Ionized Calcium.

Patient #	Total Calcium (mg/dl)*	Ionized Calcium	
		Nomogram (mg/dl)	ISE(mg/dl)
1	13.3	6.3	-
2	13.8	6.6	5.4
3	10.0	4.6	4.0
4	9.2	4.2	4.0
5	10.4	5.0	3.0
6	9.3	4.1	3.0
7	9.9	4.0	4.2
8	10.0	4.6	3.9
9	8.7	4.7	3.0
10	9.3	4.0	2.9
11	10.1	4.7	2.5
12	10.5	4.7	2.8
13	9.9	4.7	2.7
14	9.9	4.5	2.8
15	10.5	4.6	2.5
16	9.4	4.8	2.7
17	14.2	6.2	3.2

time did not permit any recovery studies for the calcium Ionalyzer® either.

(2) AUTOMATION

The results presented here for the automated analysis of plasma ammonia by ion-selective electrode represent a clear indication that automation is a partial solution to some of the problems encountered with single sample electrode systems.

(3) CORRELATION COEFFICIENT

Altnough the coefficient of correlation is of vital importance when assessing two methods, the full use of this parameter can only be utilized if there is a good reference method. Low correlation coefficients may be obtained if either the reference or proposed method possesses poor precision. It is therefore in the best interest of the analyst to select a reliable method for reference before undertaking comparative studies.

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